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Dr. Dow.

## Modified Babcock Fat Estimation

| | | |
|---|---|---|
| Solution 1 - | Amyl Alcohol - | 15cc |
| | Ethyl " | 35 cc. |
| | HCl - C.P. | 50cc. |

1. Mix well 1cc of this solution with 5cc of milk.
2. Then drop by drop add 4cc of. $H_2SO_4$ C.
3. Mix well in beaker - pour into bulb. and fill up to mark with $H_2O$. and $H_2SO_4$ equal parts.
4. Centrifuge for 5 minutes at 2000 revolutions per minute.

ggs Quantative Determination for Proteids
in Milk.

thod. (A) Dilute Human milk 1 to 10
(B) " Cows milk 1 to 20

Use Esbach's tube (reading 1 to 7 grams)

Pour diluted milk into the tube to the
mark "U" being careful to read off from
bottom of the tube.

. The phospho-tungstic acid solution
is added to mark "R".

) Place the tube in rack for 24 hrs.

. Cork tube and slowly invert 12 times
to insure thorough mixing.

. Read percentage of ppt at level of the
top of the precipitate

. Dilutions 1 to 10 percentage of proteid is
read directly from scale.

Dilution of 1-20 is multiplied by 2
" 1-5 is divided by 2.

Precipitant.

Phospho tungstic acid - 25 grams
Distilled $H_2O$. 125 cc

After thorough solution is obtained there is added HCL. (conc). 25 cc diluted with $H_2O$ (Distilled) 100 cc. This yields 250 cc of 10% solution

# CHEMICAL AND MICROSCOPICAL

# DIAGNOSIS

of phospho-tungstic acid in about 3% H.C.L. Solution is quite stable if left in dark bottle and gives satisfactory results after minutes of standing

# CHEMICAL AND MICROSCOPICAL
# DIAGNOSIS

BY

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# PREFACE TO THIRD EDITION

In the third edition such alterations have been made in the text as seem warranted, only those procedures as have had some rather extended usage being introduced. The technique of the Wassermann reaction and the preparation of vaccines has been practically rewritten at the request of a number of those who desired a somewhat more complete presentation than was given in the second edition. In these changes I acknowledge with pleasure the valuable assistance of Dr. J. Gardner Hopkins, whose practical experience with these procedures has been very extensive. The discovery of cases of sulphæmoglobinæmia has necessitated some discussion of that condition. The new antiformin method of concentrating tubercle bacilli in sputum and other fluids has now been generally accepted, and is given in full.

There is additional matter on some of the recently devised methods for determining the functional activity of the gastrointestinal tract, notably the detection of the presence of trypsin and erepsin. The dermal employment of tuberculin and the diagnosis of hydrophobia by smears made from the brain substance are introduced.

Of recent investigations on the chemistry of the urine the most practical are those of Folin in analytical methods and of Herter in the study of putrefactive processes, and such points as seem useful have been incorporated in the text. With salvarsan as a therapeutic agent the determination of arsenic in the urine is receiving attention; and the technique of the most convenient method for its detection is consequently given. The numerous fatal accidents accompanying direct transfusion have called our attention not only to the importance of hæmolytic tests on the blood but also to the possibility of serious results from intravascular agglutination and phagocytosis. Both of these questions have been discussed. Prof. Karl M. Vogel has assisted me in the revision of some of the chemical methods.

As in previous editions, I wish to acknowledge the efficient aid given by Miss S. M. Wood in the verification of references and the correction of proof.

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24778

# PREFACE TO THE FIRST EDITION

It is not necessary in the present state of medical knowledge to call attention to the importance of the results obtained by the microscopical and chemical examination of the blood and the secretions and excretions of the body, as an aid to diagnosis, for the physician is not as yet sufficiently equipped with diagnostic methods to be able to neglect the study of any phenomena which may lead to a knowledge of disease.

The facts obtained in the laboratory may occasionally be of more value than those secured by the physical examination of the patient; they more often possess a corroborative force ranking with the observations procured by the stethoscope and eye; occasionally they have merely a scientific worth and are relatively unimportant from a point of view of an immediate diagnosis.

In this volume, which represents the author's experience in hospital work and teaching of clinical pathology, the attempt is made to indicate not only the proper technique of the methods of laboratory diagnosis, but also to emphasize the relative value of the procedures and the practical importance of the knowledge so obtained. The writer hopes that the results may be of value to the students of medicine, hospital internes, and practitioners for whom this book has been written.

The author takes pleasure in acknowledging the many helpful suggestions which he owes to Professor T. Mitchell Prudden during the progress of this work and also in expressing his indebtedness to Professor P. Hanson Hiss and Dr. Charles Norris for aid in revision of several sections; to Dr. E. Libman for numerous blood slides from his large collection; to Drs. E. H. Pool and T. S. Hart for permission to reproduce specimens; and to Dr. Edward Leaming for many of the photographs. The writer's thanks are also due Miss S. M. Wood for her most valuable assistance in proof correction and in the preparation of the index.

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# PART I

# THE BLOOD

## I. GENERAL CONSIDERATIONS

THE blood is a red, slightly alkaline, albuminous fluid, which serves as a vehicle for the transfer of food and oxygen to the tissues and for the removal of carbonic acid and other waste products from the cells of the body.

The morphological elements which it contains while in the vessels are the red and white corpuscles, blood plates, and blood dust (hæmoconien). All these bodies are suspended in a fluid plasma composed of serum albumin and globulin with a small amount of fibrinogen and considerable quantities of inorganic salts, chiefly sodium chloride and sodium bicarbonate.

The red cells in health are usually considered to assume a biconcave form when not influenced by external forces; though some observers[1] have asserted that the normal figure is bell-shaped and that the disks are artificial. In blood from normal persons the red cells contain no nuclei, while in disease nucleated forms are frequently met with.

The white cells contain a nucleus under normal conditions, surrounded by a spherical cell body which often contains granules varying in size and staining reactions in the different cells. The staining reactions of these granules, largely owing to the researches of Ehrlich and his pupils, have become of great importance in the classification of the different forms of leucocytes.

The blood plates are small oval or spherical bodies without a very definite structure; but when stained they show a central mass, taking a deep nuclear stain, and a peripheral portion which takes the stain much more feebly.

The plasma of the blood also contains extremely small particles which have been called hæmoconien (blood dust) and concerning which we know but little.

In healthy persons the red corpuscles number from 4,500,000 to 5,000,000 to the c.mm.; the white from 5,000 to 10,000; the blood plates 200,000 to 600,000, though the figures for the last are not very reliable, as the plates are rapidly destroyed after the blood is shed. The specific gravity of the blood averages about 1.060. Slight variations are observed in health.

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[1] *Lewis:* Jour. Med. Research, N. S., vol. v., 1904, p. 513. [Literature.]

Within from two to eight minutes after the blood is drawn from the vessels coagulation takes place, the time depending greatly upon the amount of admixture of tissue juices with the blood and the amount of foreign material contained in the vessel into which it is drawn. When protected from foreign bodies, either by oil, or by being collected in a glass vessel which has been cleaned by the action of strong acids, the clotting is very much delayed.

The bulk of the red and white corpuscles is about half of that of the whole blood, the other half being the plasma.

The reaction of the blood is alkaline due to the presence of disodium phosphate and sodium bicarbonate, and is usually considered as equivalent to 200 to 400 milligrams of sodium hydroxide to 100 c.c. of blood. Considerable variations in the reaction may be observed under physiological conditions, such as the taking of foods, muscular work, and changes of an unknown nature, the alkalinity being least in the morning, gradually rising in the afternoon, and becoming less in the evening.

The freezing point of the blood or blood serum is quite constantly $-.55°$ to $-.57°$ C., below that of distilled water. The electrical conductivity of the serum at 25° C. is approximately $115 \times 10^{-8}$ ohms $^{-1}$.

Inasmuch as the blood occupies the position of a tissue common to all the organs of the body it may reasonably be supposed to show alterations in its composition when any of these organs undergo a change from the normal. Some of the changes in the composition of the blood, which are due either to disease or to the infectious or toxic agents which incite that condition, are quite characteristic and easily demonstrable by comparatively simple physical or chemical methods. Others either lack specific qualities or can not be made out by our present means of examination.

The changes in the morphology of the blood which characterize leukæmia, are examples of the relatively characteristic alterations of the first group; those changes in the red corpuscles which may be noted during the course of a simple anæmia, are examples of the second group—that is, of non-specific changes which are common to a large number of diseases.

The agglutinating substances which appear in the blood during the course of certain infectious diseases are examples of the alterations in composition, the nature of which are as yet wholly unknown, at least from a chemical or physical standpoint. Nevertheless, the agglutination phenomena may become useful in diagnosis, if the conditions of their occurrence in the blood in disease are carefully determined.

The facts of clinical importance which we endeavor to obtain from the blood concern chiefly the variations in the morphology of the red and white cells, the relative hæmoglobin content of the red cells, the presence or absence of micro-organisms, and the changes in the blood serum which are evidenced by the agglutination reactions. The routine examination of the blood then requires (1) enumeration of the red and white cells; (2) determination of the hæmoglobin; (3) examination of stained smears and differential count of leucocytes. If a bacterial infection is presumed to exist, a bacteriological examination of the blood may be indicated. The presence or absence of agglutination reactions is also important in febrile cases. Under special circumstances it is of interest to estimate the relative bulk of the corpuscles to the plasma, coagulation time, the iron content, the specific gravity, and the viscosity of the blood, and the freezing point and electrical conductivity of the serum.

## II. PHYSIOLOGY AND CHEMISTRY

### METHODS OF OBTAINING THE BLOOD

**Small quantities** of blood such as are required for microscopical examination in a fresh condition, or to prepare spreads for staining, are best obtained by puncturing the skin of the lobe of the ear or the tip of the finger. There are certain advantages in the use of each. The skin of the ear is less sensitive, and the instrument used in making the puncture is less obtrusive to the patient, points of no slight importance in nervous people. The ear can be used in those who are unconscious or who resist any attempt to remove the hands from under the bedclothes. On the other hand, the finger-tip, in all cases except laborers whose fingers are covered with thickened epidermis, or patients affected with marked œdema of the fingers, is more convenient and preferable because of its greater vascularity, which makes the obtaining of a large number of drops a matter of comparative ease. When repeated punctures are to be made, the same finger should not be employed for many consecutive incisions, as a very annoying anæsthesia may be produced by the severing of the small nerve-fibers of the finger-tip. In obtaining blood from infants the plantar surface of the great toe should be punctured, not the finger or ear. It is advisable to clean the skin over the point to be incised with a mixture of alcohol and ether, to remove the fat and epithelial débris always present, as these may appear in the preparation and cause the most annoying artefacts. The danger of infection is not great, yet it exists, and the writer has seen a cellulitis produced by an infected needle. It is safer, therefore, to sterilize the needle before making the puncture.

The form of needle used to make the puncture is not unimportant. The ordinary round sewing-needle is not satisfactory; it merely punctures the tissues without opening up any channel for the blood to escape. The triangular glover's needle, so often recommended, is not much better. The proper form is either what is known as a Hagedorn surgical needle, which is a flat needle with a cutting point, or a small, spear-pointed, paracentesis needle, such as is used in eye operations. Either of these instruments causes less pain than the ordinary needle, and furnishes a broad cut in the tissues, from which the blood flows freely and without the necessity of pressure, which always adds a variable amount of tissue-lymph to the blood-drop and thus vitiates the results of a hæmocytometer count. A convenient form of needle which can be obtained from dealers in microscopic supplies is shown in Fig. 1. A guard to limit the depth of the puncture is an unnecessary refinement. The skin of the finger-tip should be rendered tense by slight compression toward the periphery and the puncture made quickly and firmly, the needle going at once to the necessary depth in the soft tissues of the palmar surface

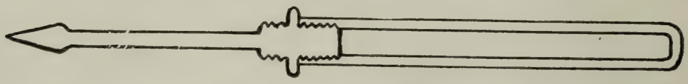

Fig. 1.—Needle for Blood Work.

of the finger-tip. The long axis of the cut should extend obliquely across the tip; not transversely, as the edges of the cut close on the slightest pressure; not longitudinally, as then fewer of the small capillary vessels are cut across and less blood can be obtained. If the ear or finger is œdematous it is advisable to remove the subcutaneous lymph as far as possible by gentle massage, the puncture then furnishing capillary blood undiluted by the tissue-fluids.

**Larger quantities** of blood, such as may be required for agglutination reactions, may be obtained by making a very deep puncture in the finger and exerting slight pressure. The lymph which is squeezed from the subcutaneous tissue by this process does not interfere with the agglutination reactions, but such blood should not be used for other tests, such as the specific gravity or coagulation reactions. The amount which may be obtained is, as a rule, not much over one cubic centimeter. For larger quantities it is necessary to puncture one of the superficial veins of the arm, the one usually selected being the median basilic. An aspirating needle of large caliber may be passed directly into the vein through the skin, after sterilization of the latter, and the blood which escapes received in a suitable vessel; or the needle may be of the size ordinarily used for hypodermic purposes, in which case it is usually necessary to attach a syringe to aspirate the blood through the needle. For such purposes the solid glass Lüer syringes are admirable, especially when furnished with needles of hardened platinum which can be sterilized in a flame. Where it is of especial importance to avoid contamination of the blood by the skin cocci, an incision should be made down to the vein that is to be punctured and the freshly sterilized needle plunged directly into the lumen of the vessel. It is often possible to obtain 10 to 15 c.c. of blood in this manner without exerting any compression on the vessels above the site of the puncture, but in persons who are deeply septic the veins may be so collapsed that it is necessary to fill them either by a compression bandage tied about the arm or by manual compression of the larger trunks. The entire operation can be carried out under local anæsthesia and the small incision in the skin left without a suture, as a sterile gauze-pad strapped over it will check hæmorrhage. The dressing need not be disturbed for a day or two, at the end of which time healing will usually have taken place.

## THE COMPOSITION OF THE CORPUSCLES AND THE SERUM

### CORPUSCLES

**The red cells** may be separated by laking with distilled water, into two bodies, one hæmoglobin, the other the stroma. The hæmoglobin is a crystalline body and has been assumed until recently to be of constant composition, but the great variations in the different analyses made by different chemists have led to the suspicion that the hæmoglobins from different animals may vary slightly in their composition and possibly the human hæmoglobins in disease differ from those in health, especially in their iron content. It has even been suggested that the hæmoglobin is merely colored by an iron-bearing chromogen and that the crystalline substance which has been regarded as of constant composition is really a mixture of two.

The stroma of the red cells consists chiefly of lecithin, cholesterin, and an albuminous body of the globulin group. It also contains potassium, sodium, calcium, magnesium, and phosphoric and hydrochloric acids. One thousand grams of red cells contain about six hundred parts of water, four hundred parts of solid matter, of which the albuminous substances and the hæmoglobin make up three hundred and eighty parts, other organic substances eight parts, the remainder is the inorganic residue. The hæmoglobin amounts to about 86 to 94 per cent. of the solid matter of the red cells.

**The leucocytes** are of slightly more complicated structure, containing the following substances:[1]

| | Per cent. |
|---|---|
| Leuconuclein | 68.78 |
| Histon | 8.67 |
| Lecithin | 4.51 |
| Fat | 4.02 |
| Cholesterin | 4.40 |
| Glycogen | 0.80 |
| Nuclein bases | 15.17 |
| Albuminous substances | 1.76 |

### SERUM

Normal serum is clear and of a pale yellow color, except after a meal rich in fats, when it may be opalescent. The specific gravity averages 1.028, with normal limits of 1.026 and 1.030. It is slightly alkaline, but less so than the plasma. It contains about 90 per cent. of water and has an average freezing point of − .56° C., but may vary between −.55° and −.57° C. The substances contained in the serum are chiefly proteids, fats, lecithin, cholesterin, and salts. Traces of urea, uric acid, creatin, hippuric acid, glucose, glycuronic acid, glycogen, and pigment are also found. Hammarsten gives the following table:

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[1] *Lilienfeld:* Du Bois Reymond's Archiv, 1892, p. 173.

| | |
|---|---|
| Total solids | 92.1 |
| Total proteids—Globulin 31.<br>Albumin 45.2 | 76.2 |
| Lecithin, fats, cholesterin, etc | 7.1 |
| Inorganic salts | 8.8 |
| Water | 907.9 |

The serum albumin of the blood appears to be a single substance except for a variation observed by Halliburton in the points of heat coagulation; that is, serum albumin $\alpha$ coagulates at 73° C.; $\beta$ at 77° C.; $\gamma$ at 85° C. The globulin, however, contains two, or possibly four groups of substances, separated by their precipitation limits with ammonium sulphate and by their solubilities in water.[1] The two main groups into which globulin may be divided are euglobulin and pseudoglobulin. Both are precipitated wholly by saturation of the fluid containing them with magnesium sulphate or by half saturation with ammonium sulphate; reactions which are used to separate them from serum albumin. The euglobulin, however, is wholly precipitated by one-third saturation, or what is more convenient, by adding to the euglobulin solution one-third of its bulk of a saturated ammonium sulphate solution. The euglobulin and the pseudoglobulin can each be separated by dialysis into a substance soluble in water, and one soluble only in dilute salt solutions, the latter being precipitated as the salts pass through the dialyzing membrane.

Especial interest in these substances is due to the fact that, as Pick and others have shown, the pseudoglobulin group has associated with it in the blood the antitoxins of diphtheria and tetanus. In other words, if blood serum containing either of these antibodies is precipitated by one-third saturation, the precipitate contains but traces of the antitoxin, while the further precipitate produced by half saturation carries down a large part of the antitoxin.

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[1] *Freund* u. *Joachim:* Zeit. f. phys. Chem., Bd. xxxvi, 1902, p. 407.

No albumose is present normally, but in leukæmia considerable quantities have been demonstrated.

Inorganic substances are chiefly sodium chloride, which is regularly present in 5.5 parts to one thousand.

Variation in the composition of the blood under physiological conditions is very slight and of no practical importance from the point of view of diagnosis. It is even very difficult to alter the relation between the water, salts, and other substances by the injection of large quantities of saline solutions or of water, the excess being rapidly excreted by the kidneys.

In anæmia there may be a reduction of the albuminous substances, which, however, does not take place to any great extent. In leukæmia, uric acid and the purin bases are often quite abundant, due to the breaking down of a large number of leucocytes. In gout also it has been shown by some observers[1] that the amount of uric acid may be increased, though others[2] deny that there is such increase.

In diabetes there is usually a hyperglycæmia, the amount of glucose rising to five or even ten grams to the thousand. Occasionally, also, the blood of a diabetic shows very large quantities of fat, so that it assumes a characteristic milky look. A moderate increase in this substance is also seen in the obese. The changes which occur in nephritis will be considered under that subject.

## THE TOTAL VOLUME OF THE BLOOD IN THE BODY

The total volume of blood has usually been assumed to be about one-thirteenth of the total body weight. The methods of determining this have been to wash the blood from the vessels by means of salt solution, and then to estimate the amount of hæmoglobin or iron contained in the washings; in this way computing the total bulk of blood in the vessels.

Haldane and Smith[3] have obtained different results by allowing a person to inhale a measured volume of carbon monoxide, and then analyzing a specimen to decide what percentage of hæmoglobin had been saturated by the formation of CO hæmoglobin.

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[1] *Klemperer:* Deut. med. Woch., 1895, p. 655.

[2] *Magnus Levy:* Zeit. f. klin. Med., Bd. xxxvi, 1898, p. 366.

[3] Journal of Physiology, vol. xxii, p. 231, 1897; vol. xxv, p. 331, 1900.

The figures obtained are from one-sixteenth to one-thirtieth of the body weight; obese persons having a much smaller relative amount of blood than those who are in good health and thin, or very muscular.

In animals only a small portion of the total mass can be obtained by direct bleeding, often not more than one-twentieth or one-thirtieth part by weight of the body.

### THE VOLUME OF THE RED AND WHITE CELLS

The hæmatocrit may be used to determine the bulk of the red corpuscles in relation to the plasma and indirectly to roughly estimate their number. The apparatus consists of a light, high-speed centrifuge, driven either by electricity or by hand, and carrying two small, graduated tubes of narrow caliber, open at each end. A large drop of blood is obtained and the tube filled by capillary action or by gentle suction through a short rubber tube provided for the purpose. The greased tip of the finger is placed over one end of the tube while the rubber mouthpiece is being removed, and the tube is quickly adjusted between the springs of the revolving arm. A similar tube filled with water or blood is placed on the opposite arm to counterbalance the weight of the blood-filled tube and allow the machine to run smoothly and without jarring. The small rubber pads which fit over the ends of the tube should be greased with vaselin to make a tight joint, as otherwise the blood will run out on the first few revolutions of the machine. Speed is necessary in all these manipulations, as the blood very quickly clots in the tube, and it is not advisable to use an oxalate or oil in the tube to prevent clotting. The only way to avoid this accident is by speed in preparation and by using only absolutely clean tubes.[1] The machine is then run for three minutes at a rate of eight to ten thousand revolutions, when the column of blood will be found to have separated into a layer of plasma and one of corpuscles, the latter occupying in normal conditions about forty-eight to fifty-two of the one hundred divisions of the tube, the lower figure being the average for women, the higher for men; in other words, under normal conditions the red cells are about half the bulk of the blood. The white cells, being specifically lighter, remain at the centripetal end of the tube as a faint gray blur, measuring only about one division of the tube. Each graduation has been found to be equivalent to about one hundred thousand corpuscles; but considerable errors are introduced in the investigation of blood from cases of severe anæmia by the variations in the bulk of the individual corpuscles from the normal and in leukæmia by the large number of leucocytes, which do not separate completely from the red cells, and hence obscure the reading of the exact point where the red column ceases. The machine is chiefly valuable as a method of determining the bulk of the corpuscles, and the relation between their number and volume, which may be designated, as suggested by Capps,[1] the "volume index." This is obtained by dividing the volume, in per cent., by the number of corpuscles, also in per cent.

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[1] See directions for cleaning hæmocytometer pipettes, p. 44.

Aspelin [2] has recently made a study of the results of hæmatocrit determinations compared with hæmocytometer estimations of the red cells and hæmoglobin determinations by the Fleischl-Miescher and Tallqvist hæmoglobinometers. The results show that the determinations offer a rough approximation to the total number of red and white cells, but that the method can by no means replace the more accurate results furnished by the Thoma-Zeiss and Fleischl-Miescher apparatus. For example, in one case of chlorosis, in which the count was 4,420,000, the hæmatocrit determination was thirty-seven, corresponding to 3,700,000 red cells, the Fleischl-Miescher showed fifty per cent. of hæmoglobin and the Tallqvist seventy per cent. The specific gravity was 1.045. The bulk of the red cells was evidently greatly diminished, as is usual in chlorosis.

## HÆMOGLOBIN AND ITS DERIVATIVES

The blood which is obtained for diagnostic purposes is usually from the small capillaries of the ear or finger, and not either pure arterial or venous blood. It therefore has characteristics belonging to both varieties. Its oxygen content is not so high as that of pure arterial blood, nor so low as that of venous blood. The color is due in part to oxyhæmoglobin, in part to hæmoglobin, for both are present in capillary blood. In arterial blood there is but little hæmoglobin and much oxyhæmoglobin; in the blood from the right heart hæmoglobin is most abundant, while oxyhæmoglobin is almost entirely absent from the blood of asphyxiated persons. The presence of oxyhæmoglobin in the circulating blood can be easily demonstrated by examining the spectrum of the light reflected from the hand, or between the closed fingers when illuminated by sunlight. If a finger is constricted the two-banded oxyhæmoglobin spectrum fades and is replaced by the single band of reduced hæmoglobin. The hæmoglobin of the blood in health is contained in the red corpuscles, ninety-four per cent. of the organic matter of the red cell being in this form of proteid, the remainder of the corpuscle being composed of an albuminous stroma, with small quantities of lecithin, cholesterin, and inorganic salts. Two views are current concerning the structure of the stroma: one that it is a spongy mass with fluid hæmoglobin in its interstices; the other that the cell is vesicular with a colorless external envelope enclosing the fluid hæmoglobin. The latter view is rendered probable by many facts observed in the study of blood pathology. When blood corpuscles are treated with a sufficient amount of distilled water, the hæmoglobin is extracted and forms a deep red solution from which the stromata may be separated by the use of the centrifuge.

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[1] Journal of Medical Research, 1903, N. S., vol. v, p. 367. An excellent study of the methods and results of the examination of blood from a number of cases of anæmia.

[2] Zeit. f. klin. Medicin., Bd. xlix, 1903, p. 393.

**Hæmoglobin** (*reduced hæmoglobin*) is a crystallizable proteid, peculiar in its large iron content, which amounts to .42 per cent. by weight of the dried substance. Chemically, hæmoglobin belongs to the group of histons, being a compound of one of the histon group, *globin*, with the iron-bearing substance, *hæmatin*. Its most important property is the power which it possesses to form a compound with oxygen, called oxyhæmoglobin, and to set free this oxygen again when the oxygen partial pressure is reduced. Upon this oxygen-carrying power is based the following test for hæmoglobin or its derivatives, such as methæmoglobin and hæmatin.

*Guaiac Reaction for Blood Pigment.*—Aqueous solutions of fresh blood when mixed with a few drops each of fresh tincture of guaiac and ozonized turpentine give a brilliant blue at the point of contact of the two fluids. This is due to the action of the blood pigment as an oxygen carrier to the guaiac. Many other substances give the same reaction: notably, unheated milk, fresh vegetables, especially potatoes, ferments, pus, and saliva. A number of inorganic salts are also capable of bluing guaiac, among them the bromides and iodides, silver, iron, and copper compounds. The reaction with copper is especially delicate, and it is important to remember that the slight coating of copper oxide which remains in a test tube after a Fehling's reaction for sugar may give rise to the guaiac reaction. The guaiac tincture should always be freshly prepared by dissolving a little powder in alcohol, but should not be stronger than four per cent., while the turpentine should be exposed for several days to light and air in a shallow dish. The reaction of the mixture should be faintly acid, using acetic acid, not an inorganic acid.

Instead of guaiac, an extract of Barbados aloin in dilute alcohol may be used,[1] the only advantage being that the aloin reaction is less influenced by an acid reaction of the blood to be tested, as, for example, in stomach contents. The color produced when blood is present is a bright cherry-red, which should appear in five to ten minutes. For further details, see under Stomach Contents and Fæces.

The deep red color of hæmoglobin makes possible its quantitative determination by colorimetric methods by comparing a solution of known dilution with a standard fluid or colored glass of the same tint. One hundred per cent. is considered normal and corresponds to fourteen per cent. of hæmoglobin by weight, which is the amount contained in normal blood; but, clinically, considerable variations are frequently seen, from eighty per cent., which is the average reading of a specimen from the females of the poorer classes, to one hundred and ten per cent., which may occasionally be seen in especially robust, healthy men. The absorption spectrum of hæmoglobin in dilute solutions is a broad band in the yellow-green portion of the spectrum, extending from a little to the red side of D nearly to E. Oxyhæmoglobin, on the other hand, shows two bands, both included between D and E, the one toward the green end of the spectrum being slightly broader and less sharply defined. (See Fig. 2, p. 13.)

**Methæmoglobin.**—When urine containing hæmoglobin is allowed to stand for a short time, the color darkens somewhat and the spectrum shows, besides bands closely corresponding to those of oxyhæmoglobin, a well-defined band in the orange between the lines C and D. As the urine ferments and the reaction becomes alkaline, the two bands corresponding to oxyhæmoglobin become more marked, while the band in the orange fades so that it may escape notice unless carefully looked for. In acid solutions four bands can be seen nearly identical with those of acid hæmatin. This altered hæmoglobin is found in small quantities in bloody urine which has been exposed to the air, or in the blood and urine of those poisoned with potassium chlorate, the nitrites, phenacetin, etc. It is most easily produced for demonstration purposes by adding to an aqueous solution of hæmoglobin a little potas-

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[1] *Schär:* Arch. d. Pharm., Bd. ccxxxviii, 1900; also *Rossel:* Deut. Arch. f. klin. Med., 1903, Bd. lxxvi, p. 505.

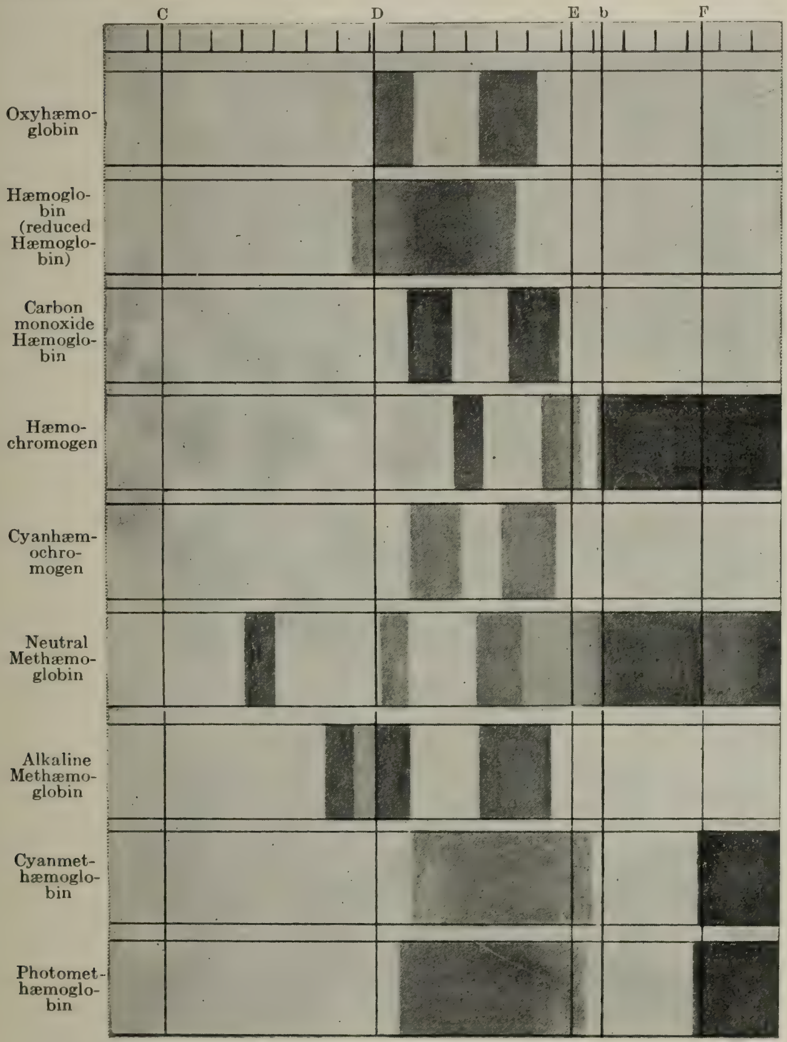

FIG. 2.—ABSORPTION SPECTRA OF HÆMOGLOBIN AND ITS DERIVATIVES.

sium ferricyanide, when the characteristic spectrum will immediately appear. The exact chemical position of methæmoglobin is not yet clear, except that it is very closely related to hæmoglobin.

**Hæmatin.**—The hæmatin component of hæmoglobin is separated from the globin by treating hæmoglobin with acids or alkalies. The resulting product differs in its spectroscopic characteristics according to whether hæmoglobin or oxyhæmoglobin is employed. In the first case, the product is known as *reduced hœmatin* or *hœmochromogen,* in the second, as hæmatin. Both of these substances are formed when blood is decomposed in the body; for example, by action of the gastric or intestinal secretions on blood which may be present in the upper portion of the intestinal tract, or by the autolytic processes which take place in old hæmorrhagic exudates, best seen in the contents of ovarian cysts into the lumen of which a considerable quantity of blood has been extravasated. Hæmatin as thus obtained is a dark brown powder or a black tarry substance, the first form being that usually seen in vomitus and old hæmorrhages; while the tarry masses are found in the stools and are composed of a mixture of hæmatin with unaltered blood or mucus. Pure hæmatin is a dark brown powder which is insoluble in water, alcohol, ether, or chloroform. It dissolves in acidulated alcohol or ether, and in dilute solutions of the alkalies. Solutions may also be obtained by the use of a saturated solution of chloral hydrate or of a 10 per cent. potassium cyanide solution, or a mixture of equal parts of alcohol and formalin.

The recognition of hæmatin and many of the medico-legal tests for blood are dependent upon the characteristic absorption spectra which are given by solutions of hæmatin, by the power which hæmatin possesses of transferring oxygen catalytically, as seen in the oxidation of resin of guaiac in the presence of ozonized oil of turpentine, and finally in its ability to form characteristic crystals with chlorine, bromine, or iodine.

(*a*) *Spectroscopic Tests for Hœmatin.*—It has been shown by Arnold[1] that there are three possible modifications of hæmatin. a neutral, an alkaline, and an acid, with spectroscopic differences.

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[1] *Arnold:* Ein Beitrag z. Spektroskopie des Blutes. Zeit. f. phys. Chem., Bd. xxix, 1900, p. 78.

The acid and alkaline varieties are of chief interest from a diagnostic aspect.

*Alkaline hæmatin* may be produced for the purposes of demonstration by heating ten c.c. of blood which has been diluted five times, with one c.c. of strong sodium hydrate. The resulting solution is at first a cherry-red, but changes to a greenish-brown. Spectroscopic examination shows a general absorption of all colors except a portion of the red end. If the solution is diluted with water, a broad, ill-defined band appears in the orange between the lines C and D, the faint border extending to the green side of the D line. The spectrum of reduced alkaline hæmatin or hæmochromogen is more characteristic, and may be produced by adding a few drops of ammonium sulphide or of Stokes's solution to the alkaline hæmatin. The spectral bands are two in number and lie in the region between the lines D and E, as do those of oxyhæmoglobin, but extending slightly toward the blue side of the E line. The band toward the blue end of the spectrum is fainter, broader, and less well-defined than that toward the red end.

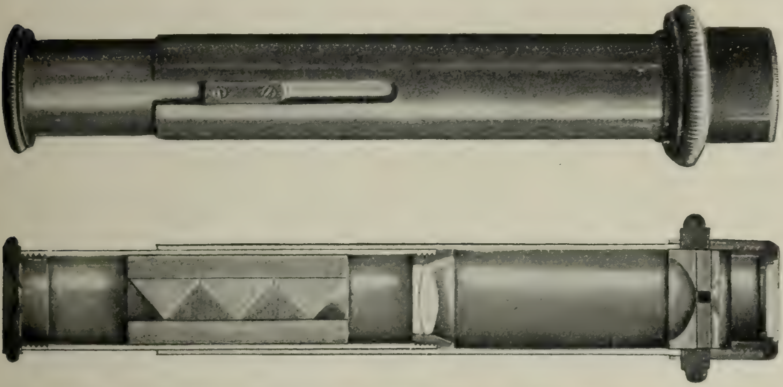

Fig. 3.—Simple form of Hand Spectroscope sufficient for all Tests given in the Text.

The spectrum of *acid hæmatin* may be obtained by shaking blood or a fragment of a dried blood-clot with alcohol or ether which has been acidulated with either glacial acetic acid or a small quantity of strong sulphuric acid. A brown tint to the extract is strong presumptive evidence of the presence of blood pigment, but it should be further tested with a spectroscope. If the ethereal extract forms an emulsion, a clear solution may be obtained by the addition of some fresh ether or of a little strong alcohol. The absorption spectrum is composed of four bands, one each in the red, in the yellow, between the yellow and the green, and between the green and the blue. Under ordinary conditions the band in the red is the only one which is well marked.

Chlorophyl, which may be present in the vomitus or the fæces, gives the same absorption spectrum so that it is well to add to the ethereal extract some alcohol and a little sodium hydrate solution and then some ammonium sulphide. Under these conditions the spectrum of hæmatin changes to that of reduced hæmatin, which gives two bands between D and E resembling those of oxyhæmoglobin, as stated above, but a little more toward E, the edge of the band toward the blue overlapping the E line. Chlorophyl does not give this change in its spectrum on the addition of a reducing agent. Occasionally, especially in old cyst contents, the hæmatin

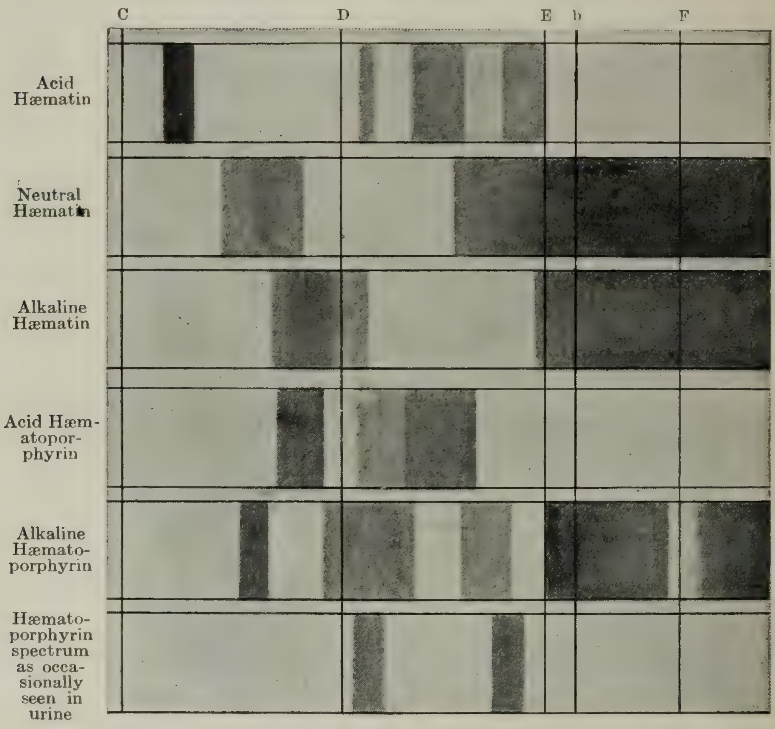

FIG. 4.—ABSORPTION SPECTRA OF HÆMOGLOBIN AND ITS DERIVATIVES.

will not dissolve in acid alcohol or ether. If so, the presence of blood pigment may be determined by dropping some of the brownish deposit into about ten c.c. of strong sulphuric acid, when if a hæmoglobin derivative be present, a purple solution of hæmatoporphyrin will be produced with characteristic spectral bands.[1] Boiling with a 50 per cent. sodium hydrate solution will also dissolve the hæmatin masses.

(*b*) *Oxidation Tests for Hœmatin.*—The method is the same as for hæmoglobin, except that as hæmatin is insoluble in water it is necessary to obtain an ethereal or alcoholic extract. If the turpentine and guaiac are then added to the acidified extract the ether will assume a beautiful purple color from the oxidation of the resin.

(*c*) *Crystallographic Tests for Hœmatin.*—One of the important properties of hæmatin is its capacity to form crystals of a characteristic morphology with the haloid elements. The crystals so formed are known as hæmin, and the test is called Teichmann's,[2] from its discoverer.

The exact composition of hæmin is still unknown. It has been generally assumed that the halogen acid was definitely combined with the hæmatin molecule to form an ester, but recently it has been shown that the acid can be largely removed from the crystals by prolonged washing, and it is therefore probably in combination with the iron of the hæmatin molecule. The technique of Teichmann's reaction is as follows: A small particle of the suspected material is placed on a slide and if moist is gently dried over a water bath or a small flame, great care being taken not to char the specimen. A small crystal of sodium chloride or iodide is added, though this addition is not usually needed in stomach contents or fæces, there being enough of the chlorides present to furnish hydrochloric acid. A drop of glacial acetic acid is then placed on the mixture and the preparation covered with a large cover glass to prevent too rapid evaporation of the acid. The slide is then gently heated on an asbestos plate or over a very small flame for some minutes, and then brought just to the boiling point, fresh acid being added as evaporation takes place. If the fluid assumes a brown color, it shows that the action of the acid has set free hydrochloric acid from the sodium chloride, and that the HCl has united with the blood pigment present to form hæmin. The acid under the cover glass is then allowed to evaporate slowly and is replaced by water or glycerin. The preparation is then examined with a power of about three hundred diameters for the small brown or black rhomboidal crystals of hæmin.

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[1] *Kratter:* Vierteljhschrift f. gericht. Med., 1892, p. 62. See also *Hammerl:* ibid., p. 44.

[2] *Teichmann:* Zeits. f. ration. Med., 1853, p. 375; 1854, p. 43; 1856, p. 141.

The test is very easy to carry out with fresh blood or with fresh hæmatin, but with old blood stains, especially those which have been in contact with iron rust, quicklime, lead, or silver salts, it is often impossible to obtain characteristic crystals. Prolonged putrefaction of the blood, and, according to the writer's experience, the addition of preservative fluids to the specimen prevents the production of hæmin, although Kobert[1] and others state that blood hardened in formalin always gives beautiful Teichmann's crystals. The fat and other substances contained in vomited matter often interfere with the test, and it may be advantageous to treat the specimen with ether, though usually some care in selecting a small blood-clot or mass of hæmatin without food contamination will obviate the difficulty. If the ordinary rapid method of heating fail, it is sometimes possible to obtain good crystals by allowing the slide to remain for some hours after the first boiling at a much lower temperature; for example, such as can be obtained by allowing the slide to rest on a steam radiator.

The difficulties which attend the production of hæmin crystals are much lessened by a method devised by Strzyzowski.[2] The reagent, which should always be freshly prepared, contains glacial acetic acid, water, and alcohol, one c.c. each, to which is added three to five drops of hydriodic acid of a specific gravity of 1.5. The substance to be tested is dried on a slide and covered with a cover glass. The reagent is then added, and the slide heated over a small flame for about ten seconds. The fluid should be replaced as fast as it evaporates and brought just to the boiling point. The heating may be repeated if no crystals be obtained at the first trial. The crystals formed are much darker than those containing chlorine, and those from old blood are very small and need a power of three hundred diameters for their recognition. The test is a more delicate one for blood than the Teichmann test, as it will show one two-thousandth of a milligram, while the older method ceases to give constant results at one four-hundredth of a milligram of blood.

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[1] *Kobert:* Wirbeltierblut in Mikrokristallographischer Hinsicht, Stuttgart, 1901, p. 55. *Browicz:* Virch. Arch., Bd. clxii, 1900, p. 373.

[2] *Strzyzowski:* Therap. Monatshefte, 1902, p. 459.

**Carbon Monoxide Hæmoglobin.**—The hæmoglobin of the blood is capable of uniting with other gases beside oxygen, notably carbon and nitrogen monoxides. The compounds so formed are much less easily dissociable than oxyhæmoglobin; and therefore, in poisoning by these gases, the resistant compound takes the place of the respiratory oxyhæmoglobin, and death may result from what is practically suffocation due to lack of oxygen in the circulation. It is possible to remove the CO or NO from its combination with the hæmoglobin by shaking for some time with free access of air, or in the human body, by artificial respiration. The color of the blood of persons suffering from acute poisoning after the inhalation of large quantities of illuminating gas, or the gases from stoves, or fumes of charcoal, is a distinct cherry-red, and this change is to be noted in both the venous and arterial blood.

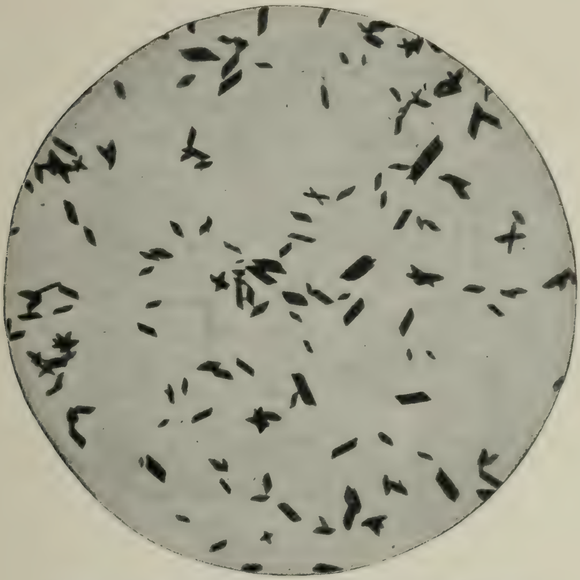

FIG. 5.—HÆMIN CRYSTALS. STRZYZOWSKI METHOD.

A rapid diagnosis can sometimes be made by comparing a drop of the patient's blood with a drop taken from a normal person and observing the difference in color, but it is usually necessary to resort to chemical tests, for small quantities of CO do not cause sufficient alteration in the color to permit of a diagnosis; and it is also important to remember that the CO-hæmoglobin may disappear from the blood in a few days or even hours, so that a prompt examination should always be made. Occasionally, however, the CO-hæmoglobin persists for as long a period as a week, and it can often be demonstrated in the blood after death.[1]

The more reliable tests are the following:

(*a*) *Spectroscopic.*—The absorption spectrum of CO-hæmoglobin resembles that of oxyhæmoglobin, except that the two bands are shifted slightly toward the violet end of the spectrum, and that they do not become fused into a single band when the solution is treated with a reducing agent such as ammonium sulphide or Stokes's reagent. The method is as follows: Obtain a small quantity of blood by puncturing the finger, and dilute with ten volumes of distilled water. Then add considerable quantities of the reducing agent, cork the test tube, and after the solutions have stood for a few minutes examine for two bands between D and E, using a faint illumination. The position of these bands should be compared with pure CO-hæmoglobin, which can be easily prepared by passing illuminating gas for two minutes through a few cubic centimeters of diluted blood in a test tube. Small quantities of CO-hæmoglobin can not be detected by this method, for the dark band of reduced hæmoglobin obscures the two faint bands of the CO-hæmoglobin and prevents their recognition. The limit is about 20 per cent. of CO-hæmoglobin; smaller amounts than this can not be detected in the blood by the spectroscope.

(*b*) *Chemical.*—(1) Sodium Hydrate Test.—The blood is diluted with twenty parts of water, and an equal bulk of a 30 per cent. solution of sodium hydrate is added. If the blood contains any CO-hæmoglobin, the precipitate formed will be first whitish and then bright red. A control tube of normal blood gives a dirty brown precipitate.[2] After standing for some time the characteristic color disappears.

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[1] A valuable monograph on this subject is *W. Sachs:* Die Kohlenoxid-Vergiftung, Braunschweig, 1900.

[2] *Salkowski:* Zeit. f. phys. Chemie., Bd. xii, 1888, p. 227.

(2) Tannin Test.[1]—The blood to be tested for CO-hæmoglobin is divided into two portions and diluted with four parts of distilled water. The two solutions are then placed in two small flasks or large test tubes, and twenty drops of a 10 per cent. potassium ferricyanide solution are added to change the hæmoglobin into methæmoglobin. Both solutions are allowed to stand for a few minutes, and then both are corked and one is shaken thoroughly for ten to fifteen minutes, occasionally removing the cork to allow free access of air. A few drops of yellow ammonium sulphide are then added to each flask, five to ten are usually sufficient. Now ten c.c. of a 10 per cent. solution of tannin are added to each flask. The one from which the CO-hæmoglobin has been removed by shaking will show a dirty olive-green precipitate, while the flask which has not been shaken and still contains CO-hæmoglobin will show a bright-red precipitate which is characteristic of CO-hæmoglobin. This is a more delicate test than that with the spectroscope and will give a reaction when the blood contains only 5 per cent. of CO-hæmoglobin.

(3) Test of Katayama.[2]—One c.c. of the blood to be tested is diluted with fifty c.c. of water. Ten c.c. of this solution are mixed with .2 c.c. of yellow ammonium sulphide solution and then .2 to .3 c.c. of 30 per cent. acetic acid added, or a sufficient quantity to make the reaction faintly acid. The contents of the tube are then mixed by inverting it once or twice. Normal blood gives a grayish green color. If 20 per cent. of CO-hæmoglobin, or, in favorable cases, 15 per cent., be present, the fluid will show a more or less marked rose color, and in twenty-four hours a reddish precipitate.

The air of the rooms suspected to contain CO can be tested by exposing strips of filter paper which have been soaked in a neutral solution of palladium chloride and dried. The solution should contain 2 milligrams of $PdCl_2$ to one hundred c.c. of water. Before exposing, the strip should be moistened. Air containing two parts of CO in ten thousand will blacken such a test paper in a few minutes. The test is of especial importance in poisoning from stove gases where there is no odor to guide in the diagnosis.

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[1] *Wachholz* u. *Sieradzki:* Zeits. f. Medizinalbeamte, Bd. x, 1897, p. 269.
[2] Virchow's Archiv, Bd. cxiv, 1888, p. 53.

**Cyanogen Compounds.**—In testing for blood in old stains. when the insoluble hæmatin is dissolved by means of potassium cyanide, the solution thus obtained gives an ill-defined, broad band in the yellow-green portion of the spectrum extending from λ 578 to λ 527 or to E. This spectrum is that of cyanhæmatin. When this solution is reduced by ammonium sulphide, cyanhæmochromogen is produced, with a spectrum whose bands resemble very closely those of hæmochromogen but are more toward the red and do not touch the E line. The wave lengths of the two bands correspond to λ 577–562 and λ 548–532. In cases of poisoning by hydrocyanic acid or a cyanide, the blood pigment in the walls or lumen of the stomach shows this spectrum when the solution is reduced by treatment with ammonium sulphide.[1]

**Sulphæmoglobin.**—Hæmoglobin combines with hydrogen sulphide, the blood giving the spectrum of oxyhæmoglobin plus a broad band in the red, extending from λ 610 to λ 625 and seen only in very concentrated solutions or by direct spectroscopic observation through the hand held up to a strong light. A number of cases of sulphæmoglobinæmia apparently due to intestinal putrefaction have been reported.

**Hæmatoporphyrin** is a derivative of hæmoglobin which contains no iron and forms a dark brown powder insoluble in water, but solutions can be obtained by the use of strong alkalies or acids. It is chiefly of interest in connection with its appearance in the blood and urine in poisoning by sulphonal, and its identification will be treated of under that head. Acid hæmatoporphyrin is easily obtained for the purpose of studying its absorption spectrum, by adding five drops of blood to ten c.c. of strong sulphuric acid.

The demonstration of the spectrum of hæmatoporphyrin is of great value for the medico-legal identification of blood. Fragments of blood which have been exposed to sunlight or which have been heated or dried for a long time are often with difficulty soluble in water, alkalies, or potassium cyanide. In such cases, if a small fragment of the suspected material, mixed with a few drops of concentrated sulphuric acid, be crushed between two thin glass slides and the transparent fluid examined either with a direct vision spectroscope or by a micro-spectroscope, it is often possible to demonstrate the bands of hæmatoporphyrin when other methods for the recognition of blood fail.[1] If the blood is mixed with a large quantity of organic matter, such as paper, shavings, or is on walls or in earth, the dark solution which results from the charring caused by the acid will obscure the spectral bands. Ipsen[2] has suggested the addition of fresh acid or dilution of the solution with acid. This, however, is not always successful. Ziemke[3] has therefore suggested the following modification: The suspected material is pulverized, and after mixing with a few c.c. of concentrated sulphuric acid is allowed to stand for twenty-four hours. The dark liquid is filtered through glass wool or asbestos, which requires considerable time as the fluid is thick. The filtrate is poured into a large quantity of distilled water and neutralized with strong ammonia. A brown precipitate of hæmatoporphyrin is formed which rapidly settles and is washed several times by decantation. The dried residue is dissolved in a mixture of equal parts of absolute alcohol and strong ammonia water and filtered. The clear brown fluid shows the four-banded spectrum of hæmatoporphyrin in an alkaline solution. By treatment with sulphuric acid the spectrum of hæmatoporphyrin in acid solutions can be produced, but it is faint and not so easily seen as the alkaline spectrum.

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[1] *Ziemke* u. *Müller:* Arch. f. Anat. u. Phys., Supplement-Band, 1901, p. 177; also *Marx:* Viert. f. ger. Med., Bd. xxvii, 1904, p. 300.

**Hæmatoidin** is a substance found in old blood-clots, in hæmorrhagic exudates, especially in thyroid and mammary cysts, in sputum and in fæces when there has been transudation of blood into the lung or intestine. It is identical with bilirubin and gives the color reactions of that substance. It occurs in small irregular masses, in rhombic plates, or in bundles of needles. Hæmatoidin is derived from the hæmoglobin of the blood by changes which can be well observed in a subcutaneous exudate, such as an ecchymosis.

### QUANTITATIVE ESTIMATION OF HÆMOGLOBIN

**Fleischl Hæmoglobinometer.**—The instrument most often used for this purpose is that devised by Von Fleischl, which is quite sufficient for all clinical purposes. Greater accuracy can be obtained by using certain modifications of the apparatus, as suggested by Miescher.

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[1] *Kratter:* Viert. f. ger. Med., Bd. iv, 1892, p. 62.

[2] Viert. f. ger. Med., 1900, Bd. xx, p. 1.

[3] *Ziemke:* Forensischen Blutnachweis. Preus. med. Beamt. Zeit., 1900; also Viert. f. ger. Med., Bd. xxii, p. 231, 1901.

The simple form of the Fleischl model will first be described. It consists essentially of a cell to contain the hæmoglobin solution, which can be placed over a circular hole on the stage of the instrument, and a colored glass wedge for comparison with the diluted blood. Light from a candle or small gas flame is reflected by a plaster of Paris reflector through a cell. The latter has two divisions—one to be filled with the diluted blood; under the other passes the wedge of red glass so arranged as to be movable along a scale by means of a rack and pinion. The diluted blood and the standard glass scale are thus directly compared and the amount of hæmoglobin read off from the scale, which is calibrated in percentages.

To use the instrument, fill both sides of the cell about half full of *distilled water* or $\frac{1}{10}$ per cent. sodium carbonate solution. (Occasionally, when using sodium carbonate solution as a diluting fluid, the resulting mixture will not be transparent; this is due to the presence of bicarbonate in the solution.) The small capillary pipette is then filled with blood. As a rule, this is easily done by simply touching the end of the pipette to the drop on the end of the finger, but if the inner surface is not perfectly clean the blood may refuse to run in; in this dilemma, if the end of the pipette be kept inside the drop and the open end of the tube gently tapped against the finger, the blood will be pushed into the capillary by a few strokes. The capillary tube should be carefully filled, so that the blood is just flush with the end of the tube. The column should be neither convex nor concave. If too full, wipe off with the dry finger. Blotting paper should not be used, as it removes serum and leaves the corpuscles—i.e., concentrates the blood in the tube. The outside of the tube should also be carefully cleaned from any adherent blood, and then the pipette dipped into one compartment of the chamber and rapidly shaken back and forth to wash out the blood. If any clots are seen floating about in the fluid, the whole process should be again carried out.

Wash out the pipette into the cell by forcing a few drops of water through it with a medicine-dropper, and with the latter fill up both sides of the chamber with water or sodium carbonate solution. The cell should be exactly filled and then stirred with the pipette handle in order to thoroughly mix the hæmoglobin solution. Neither a convex nor a concave meniscus should be present on the surface of the fluid in the cell.

After the latter has been placed in the aperture on the stage, the light is adjusted so that it falls on the mirror—not from in front, but from either the right or the left. The adjustment of the mirror to this position sometimes necessitates loosening the screw by which it is held. The reason for this position of the mirror is that the light reflected through the two halves of the cell should come from exactly the same distance on the mirror and from an equally illuminated surface.

When used in the ordinary way, with the mirror facing the front of the machine, the glass wedge obtains its light from the upper and posterior surface of the mirror, which may be half a centimeter nearer than that which furnishes light for the blood-filled side of the cell, whereas by receiving the light on a surface whose direction is that of the long axis of the wedge each half of the field is illuminated by light from equidistant points. This precaution is necessary, however, only when very exact results are desired.

The cell should also be so arranged that the light from each half strikes the two halves of the retina; that is, we should face the light, for, though the two lateral halves of the retina are unequally sensitive, yet the upper and lower halves are relatively even more unequal, and by alternating eyes, we can examine each half of the field with the nasal retinal field in one case and the temporal in the other, thus diminishing the error.

The whole apparatus should be either in a dark room or in a blackened box with a curtain to keep out any extraneous light. To avoid the glare of the candle it is well to use a short pasteboard tube over the cell and look directly through it. Use only a moderately bright illumination. Judgment for red is very variable in different people, and the color rapidly fatigues the retina. The best way to obviate errors from these sources is to use little light and to alternate in the use of each eye; or, what is better, to use both eyes and rest them every few seconds, preferably by looking at something green, the complementary color to red, thus increasing the sensibility to the latter color. The milled head should be moved by quick turns, never slowly, as then the gradual change in the color is lost to the tiring retina. A quick turn, on the other hand, gives a rapid change of color, and through a considerable range, so that the eye readily notes the alteration in color depth. Narrow the field of the instrument by a piece of cardboard or blackened brass, with a slot cut in it about four millimeters in diameter.

In case of low readings, use two or even three pipettes full and divide the result by two or three. With all possible precautions the errors incident to the measurement of the blood in the capillary and the reading of the scale, which is accurate only for a limited central portion of the wedge, amount to at least 5 per cent. A low retinal color sensibility for red may add 5 per cent. more.

**Fleischl-Miescher Hæmoglobinometer.**—To avoid certain of these errors, Miescher has so modified the original Fleischl hæmoglobinometer as to make it an instrument of great precision. Its disadvantages are that it is more troublesome to use, is more bulky, and more expensive than the other form.

The main points of improvement are as follows:

Special care is taken in the selection of the glass color wedge, so that, under proper conditions of illumination, it may correspond as closely as possible in color to the diluted blood. The inaccuracy inherent to the use of the small capillary pipette is obviated by employing a mixing pipette similar to that used with the Thoma-Zeiss hæmocytometer. By the use of this pipette, small errors, such as arise from any inequality in the concentration of the solution in the cell or the formation of small clots about the ends of the capillary tubes, are avoided. The graduation of the pipette is also an improvement on the older method of divisions from .1 to 1. The marks are $\frac{1}{2}$, $\frac{2}{3}$, and 1, corresponding to dilutions of 1 to 400, 1 to 300, and 1 to 200. Above and below each of these main divisions are two marks, each corresponding to one one-hundredth of the contents of the capillary tube. If now the blood column should be drawn up a little too far, or not far enough, the distance can be read off and the final result increased or diminished by a corresponding amount, thus saving much time in the adjustment of the blood column. The tip of the pipette is polished so that the lower end of the column of fluid can be seen, a frequent source of error being the removal of a millimeter or so of the blood in the lower end of the capillary while cleaning off the extraneous blood preparatory to drawing up the mixing fluid. As now we have blood of a fixed dilution, the small cells can be filled with the mixture and capped with glass plates, regardless of the loss of a few drops of the fluid, while in the old method any loss in the fluid contents of the cell invalidated the result. Two cells are provided, one 15 millimeters high, the other 12 millimeters or four-fifths of the first. Both of these can be filled from the contents of a single mixing pipette, and by reading each, a different portion of the scale is used and another source of error removed. It is best to make ten readings from a given specimen of blood and take the mean of the result.

If a large number of leucocytes be present, the fluid may be turbid and the exact readings in doubt. In such a case, the diluted blood should be centrifuged to remove the leucocytes before filling the chambers. With care and good color vision, the readings on this improved instrument can be relied on as accurate to within 1 per cent. A table goes with each instrument which gives the absolute amount in milligrams of hæmoglobin corresponding to a given point on the scale, so that instead of using the rather indefinite percentages based upon average normal blood, the results may be given in grams of hæmoglobin per one hundred c. c. of blood. For the accurate scientific esti-

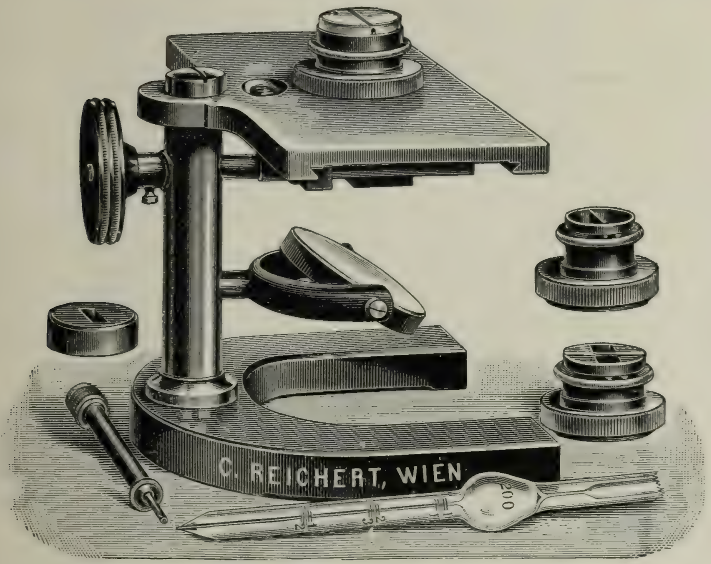

Fig. 6.—Fleischl–Miescher Hæmoglobinometer.

mation of the hæmoglobin content of the blood this instrument is invaluable.[1]

**Gowers's Hæmoglobinometer.**—This cheap and simple instrument is much used in rough clinical work, and while its results are often inaccurate to 15 per cent., yet its compactness and ease of working have contributed to the favor with which it is still regarded by many clinicians. It consists of a small glass tube containing a standard picrocarmine solution of such a depth that it corresponds to a 1 per cent. solution of normal blood. A comparison tube graduated in ten parts is used as a container for the diluted blood. Twenty cubic millimeters of the blood are measured off by a pipette and blown into the graduated tube, which should contain a little water. The standard tube and the comparison tube are then held before a piece of white paper and water gradually added from a medicine-dropper until the blood column to be tested is of the same color as the standard. The

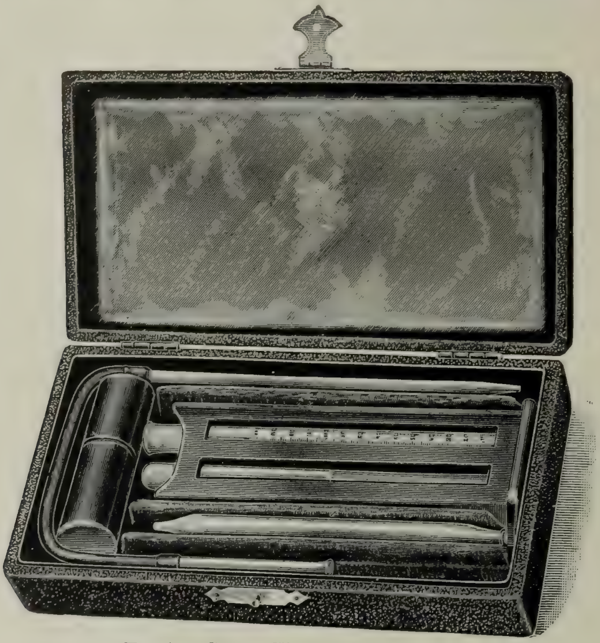

Fig. 7.—Sahli Hæmoglobinometer.

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[1] See *Jaquet:* Corr.-Bl. f. Schw. Aerzte, 1897, pp. 129 and 164; and *Veillon:* Arch. f. Exp. Path. u. Pharm., Bd. xxxix, 1897, p. 385.

per cent. of hæmoglobin is read off from the graduations of the tube; if the column stands at the mark 100, then the amount of hæmoglobin is normal. A serious objection to this instrument is that the color of the standard tube is not permanent but must be renewed after a year or so.

**Sahli Hæmoglobinometer.**—An improved form of the Gowers hæmoglobinometer has been recently devised by Sahli.[1]

The comparison fluid which accompanies the apparatus is made up by mixing twenty cubic millimeters of blood containing a normal amount of hæmoglobin with ten volumes of decinormal hydrochloric acid, and diluting so that the solution has a concentration of 1 per cent. This fluid is sealed in a small glass tube which is contained in a hard-rubber stand with two compartments and a ground glass screen at the back, so that the mixing tube is placed under exactly similar conditions as the standard solution. The transparent brownish fluid which is thus obtained is quite permanent, and as the blood to be tested is compared with a solution of the same tint the accurate estimate of the depth of color is much more easy than in the original form of the Gowers instrument.

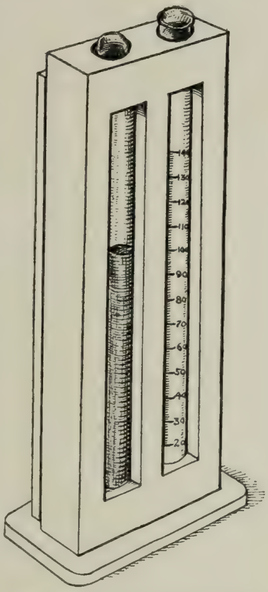

FIG. 8.—STAND FOR COMPARISON TUBES OF THE SAHLI HÆMOGLOBINOMETER.

Twenty cubic millimeters of the blood to be tested are placed in a small mixing tube to which decinormal hydrochloric acid has been added to the mark 10 by means of a pipette. The blood remaining in the pipette is to be thoroughly removed by sucking up the acid into the pipette and repeatedly blowing out the mixture until all of the solution is removed. In a few moments the acid mixture becomes clear and of a dark brown color. Tap water is then added to the graduated tube until the color of the blood mixture corresponds exactly with the standard solution of the acid hæmatin contained in the comparison tube. When this point is reached the height of the fluid is read off in the mixing tube, thus giving the hæmoglobin in percentages as compared with a standard quantity of normal blood.

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[1] Verh. Congress für Innere Medizin, 1902, p. 230.

It is better in using the apparatus to turn the mixing tube so that the graduations are not visible, as it is then much easier to compare the two colors. The readings may be made either in daylight or with any sort of artificial light. If special accuracy is desired, the eye must be screened from extraneous illumination while the dilution of the fluid is being made.

It is convenient to employ two mixing tubes and to make two parallel dilutions, the first giving approximate results, then if too much water has been added to the first, the second can be diluted more slowly to the exact point. This obviates the necessity of a second puncture of the finger.

**Oliver Hæmoglobinometer.**—This instrument has been devised to overcome certain unavoidable errors in the original form of the Fleischl hæmoglobinometer, and is an accurate and compact instrument ranking next to the Fleischl-Miescher. The apparatus can be obtained with color scales which can be read in daylight, and though this is less accurate than the instrument adjusted for candle-light, yet it is quite sufficient for clinical purposes. The blood is obtained in a small capillary pipette containing 4.7 cubic millimeters, a much more practical form than the Fleischl, as it is made of heavy glass tubing with a large bore so that it can be easily cleaned by passing a needle threaded with darning cotton through it. The mixing pipette is provided with a short piece of rubber tubing on its tip which slips over the end of the capillary pipette and thus may be used to wash out all blood into the comparison cell. The dilution should be made with distilled water or a $\frac{1}{10}$ per cent. solution of sodium carbonate.

The comparison cell is then covered with a slip of glass of a pale blue color in order to diminish the yellow tint of the blood and make it correspond more closely with the bluish pink of the standard disks. These disks are arranged in a metal holder and rise by steps corresponding to 10 per cent. of hæmoglobin, the lowest being 10 per cent. and the highest 120 per cent. To exclude extraneous light a small collapsible tube is provided with two apertures at its lower end through which the colors of the standard disks and of the blood to be examined can be compared. The disks are first moved along parallel with the blood mixture to be tested until an approximate agreement is reached. To make readings closer than 10 per cent., a series of riders is furnished, which permit determinations by stages of 2½ per cent., or if special accuracy is required, by 1 per cent. For clinical use one rider is sufficient—namely, that corresponding to 5 per cent. This enables one, by superimposing the rider on the disk, to obtain a color 5 per cent. above that of the standard overlaid.

**Tallqvist Hæmoglobinometer.**—Tallqvist[1] has recently revived an old method of determining the amount of hæmoglobin in the blood by publishing a color scale corresponding to the various amounts of hæmoglobin, each shade in the scale being 10 per cent. higher than the one preceding it, the darkest color being regarded as normal and estimated at 100 per cent. The blood is dropped on a small sheet of special filter paper, a book of which is bound up with the scale, and after the glazed surface has disappeared by the blood soaking into the paper, the spot is compared with the various shades of the scale until the color is approximately matched. The per cent. corresponding to this portion of the scale is the amount of hæmoglobin in the blood. As a large number of observations can be made by this method in a very short time, and as no special apparatus is required and the readings are made in daylight, it is excellent for rapid estimations of the hæmoglobin as a preliminary to more accurate methods if a marked anæmia be present. The error of this process is not less than 10 to 15 per cent., and seems to be much more dependent upon the ability of different individuals to judge shades of color than with the Fleischl. Some persons can not obtain even approximately accurate results; others read more closely than with the more elaborate forms of apparatus. The color of the tinted standard areas also varies a good deal, being much lighter in the books recently lithographed as compared to those originally sent out in 1900, or the scale as published in the original article. It is evident that while this simple method is valuable in determining the presence or absence of an anæmic condition of the blood, it can not replace the more accurate instruments in determining small variations in hæmoglobin, which are of importance in connection with the diagnosis and treatment of the diseases of the blood.

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[1] Zeit f. klin. Med., 1900, Bd. xl, p. 137.

**Dare's Hæmoglobinometer.**[1]—This instrument differs from others in general use in the fact that undiluted blood is directly compared with a colored glass wedge. The blood to be tested is allowed to run in between two small glass slides held in a clamp, and there forms a thin layer. The wedge pipette carrying the layer of blood is then inserted in the proper slot in the apparatus. The focus of the eyepiece is adjusted, the candle which is attached to the instrument is lighted, and the wedge is rotated by means of a milled head until the circular area corresponding to the blood matches exactly the color of the circular area past which the wedge of colored glass rotates. It is not necessary to use the instrument in a wholly darkened room, but merely to avoid strong direct light. The instrument is very useful in rapid determinations of hæmoglobin, but in the writer's hands has not proved as satisfactory as the Fleischl-Miescher or the Oliver instruments. The readings have been lower than those obtained by the other methods, though Simon[2] states that in his experience they are higher.

## THE IRON CONTENT OF THE BLOOD

The estimation of the iron content of the blood for clinical purposes is accomplished by means of an apparatus known as Jolles's ferrometer. The apparatus is accompanied by full directions for carrying out the technical portion of the procedure, and also by the standard reagents.

The principles of the method are the oxidation and the solution of the iron of the blood by heating a measured quantity in a platinum crucible with potassium bisulphate. When completely oxidized, the contents of the crucible are washed out with hot water and mixed with one c.c. of 30 per cent. hydrochloric acid and four c.c. of ammonium sulphocyanide solution containing 7.5 grams to the liter. This solution is red and the amount of iron present is determined by a Fleischl-Miescher hæmoglobinometer. One hundred grams of normal blood contain 0.075 gram of $Fe_2O_3$ or 0.0525 gram of Fe.

The results of an elaborate series of experiments comparing the ferrometer with the Fleischl-Miescher hæmoglobinometer[3] have shown that the values obtained by these two instruments do not regularly correspond. It is possible that the hæmoglobin of the blood contains a variable amount of iron, or that there are substances in solution in the blood which alter the depth of color obtained in using the hæmoglobinometer. Both instruments give constant results on the same specimen of blood, but when applied to chlorotic blood, for example, the ferrometer may give 50 per cent. of the normal, while the hæmoglobinometer gives lower results, in some cases as low as 20 per cent. In other cases of the same disease the hæmoglobinometer gives much higher results than the ferrometer. The same variation has been found in pernicious anæmia and diabetes, the iron having been found higher than the hæmoglobin by some observers and lower by others. In other words, the ferrometer can not replace the hæmoglobinometer in our present state of knowledge, though it remains an instrument capable of furnishing scientific facts of great interest. The diagnosis of the various types of anæmia must depend more upon the results of the hæmoglobinometer than upon those obtained by the ferrometer, because the mass of clinical observations on the blood in anæmia which have accrued in the past twenty years are expressed in terms of hæmoglobin.

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[1] Philadelphia Med. Journal, 1900, vol. vi, p. 557.

[2] Clinical Diagnosis, 1904.

[3] *Rosin* u. *Jellinek:* Zeit. f. klin. Med., 1900, Bd. xxxix, p. 109.

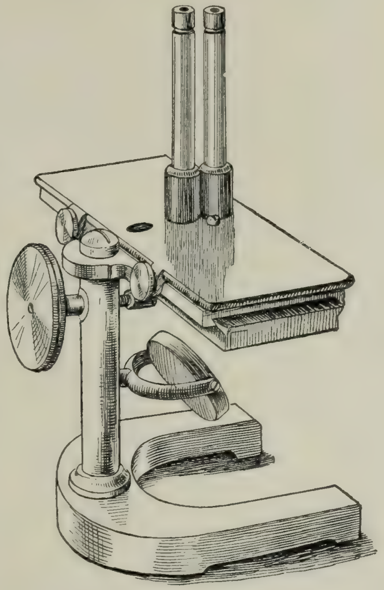

Fig. 9.—Jolles' Clinical Ferrometer

#### DETERMINATION OF THE SPECIFIC GRAVITY OF THE BLOOD

For clinical purposes two methods have been devised, one of which gives accurate results with very small quantities of blood.

**Schmaltz's Method.**—A small tube of thick glass, both ends of which are open and carefully polished, is first weighed and then filled with distilled water at 18° C., and the weight of the water determined. Finally, after the tube is again dried, it is filled with blood obtained either from a vein or from a very deep puncture in the finger; the ends of the tube are carefully cleaned off from an excess of blood, and the tube and its contents accurately weighed in a balance. For accurate results the tube should contain at least two decigrams of blood and the weighing should be to a tenth of a milligram. The specific gravity of the blood is obtained by dividing the weight of the blood by the weight of the water at the same temperature. Evidently this method is not suited for ordinary clinical work and can only be carried out in hospitals or laboratories.

**Hammerschlag's Method.**—For the practitioner Hammerschlag's method gives relatively accurate results and can be carried out in a few minutes. The apparatus needed is a clean and dry urinometer jar and some benzol and chloroform. The jar is first filled with a mixture of the two in such proportions that the specific gravity is about 1.045 to 1.050.

The finger is then punctured and a medium sized drop obtained (no pressure) and conveyed to the cylinder by means of an ordinary medicine-dropper, or shaken off directly from the finger into the fluid. Too small a drop does not rise or fall quickly in the fluid; too large a one is easily broken up during the mixing process. If the drop sinks, add more chloroform; if it rises, add more benzol. When it comes to rest and remains in the same position for half a minute after the top of the jar has been covered to prevent convection currents due to evaporation of the benzol chloroform mixture, the specific gravity of the drop is the same as that of the fluid and the specific gravity of the mixture is easily found by the float. The latter is usually graduated only between 1.000 and 1.060, and sometimes the gravity of normal blood will be found to exceed that figure, but, for anæmic conditions, 1.060 is sufficient.

Certain precautions are necessary. The jar should either be carefully stirred after each addition of either constituent, or the whole jar should be carefully inverted after closing the top with the palm of the hand. The drop should not contain any air, and the whole process should be done quickly. The normal average specific gravity for men is 1.059; for women, 1.056.

The errors of the method are due in the first place to the fact that the ordinary urinary specific gravity floats are graduated for an aqueous fluid and not for a chloroform and benzol mixture which, owing to its low surface tension, gives higher readings on the scale than does water.[1] Either an hydrometer corrected to a chloroform and benzol mixture must be obtained, or the one used must be corrected by making a mixture of chloroform and benzol of such a strength that a drop of distilled water remains suspended in it. Its specific gravity is therefore 1.000. The hydrometer is then placed in this mixture and the point at which it comes to rest is noted. This will usually be four or five units in the third decimal above the zero point as determined in water. With a good instrument this difference is constant and may be used to correct further readings at other points on the scale; but with some hydrometers the scale varies slightly and the readings are quite irregular and can not be corrected. In such cases it is always better to use a Westphal balance, which gives rapid readings correct to the fourth decimal.

The interest in the specific gravity lies chiefly in the fact that it furnishes an indirect means of estimating the hæmoglobin content of the blood, for it has been shown that within certain limits, the variation in hæmoglobin causes a corresponding variation in specific gravity, and the table which is appended shows the results obtained by Hammerschlag with this method. Assuming that the determination of the specific gravity has been accurately made, there are still other sources of error, among which are normal variations due to food, exercise, etc., these lie within .003. Nephritis alters the specific gravity of the serum. In circulatory diseases, which introduce a number of factors dependent upon the increased number of red cells seen under these conditions, the results are often incorrect. Leukæmia, in which the specific gravity is slightly higher than would be expected from the red cell count, due to the increase in the white cells; and pernicious anæmia, in which the high color index of the individual cells adds 2 per cent. at least to the value of the result obtained by gravity, give inaccurate figures. In such cases the hæmoglobinometer must be used. The use of the method, however, has been rendered less important to the practitioner since the introduction of the Tallqvist color book for determining hæmoglobin, inasmuch as the latter gives equally accurate results which can be obtained much more easily than the specific gravity.

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[1] *Baumann:* British Med. Journal, 1904, p. 473, and *Levy:* Proc. Royal Soc., 1902, vol. lxxi, p. 171.

**Hammerschlag Method.**

| Specific Gravity. | Hæmoglobin. (According to Fleischl.) | |
|---|---|---|
| 1.033–1.035 | 25–30 | per cent. |
| 1.035–1.038 | 30–35 | “ |
| 1.038–1.040 | 35–40 | “ |
| 1.040–1.045 | 40–45 | “ |
| 1.045–1.048 | 45–55 | “ |
| 1.048–1.050 | 55–65 | “ |
| 1.050–1.053 | 65–70 | “ |
| 1.053–1.055 | 70–75 | “ |
| 1.055–1.057 | 75–85 | “ |
| 1.057–1.060 | 85–95 | “ |

**Schmaltz Method.**

| Specific Gravity. | | Hæmoglobin. | | |
|---|---|---|---|---|
| 1.030 | = | 20 | per cent. | ± |
| 1.035 | = | 30 | “ | “ |
| 1.038 | = | 35 | “ | “ |
| 1.041 | = | 40 | “ | “ |
| 1.0425 | = | 45 | “ | “ |
| 1.0455 | = | 50 | “ | “ |
| 1.048 | = | 55 | “ | “ |
| 1.049 | = | 60 | “ | “ |
| 1.051 | = | 65 | “ | “ |
| 1.052 | = | 70 | “ | “ |
| 1.0535 | = | 75 | “ | “ |
| 1.056 | = | 80 | “ | “ |
| 1.0575 | = | 90 | “ | “ |
| 1.059 | = | 100 | “ | “ |

## DETERMINATION OF THE SPECIFIC GRAVITY OF THE SERUM

The specific gravity of the blood serum under normal conditions varies from 1.028 to 1.030.

The specific gravity of the serum may be obtained in the same manner as that of the whole blood, by allowing a number of drops of that fluid to coagulate in a small bottle, and when, at the end of twenty-four hours, the serum has separated, a drop of this fluid is pipetted off and allowed to fall into the chloroform benzol mixture.

## ENUMERATION OF THE RED AND WHITE CELLS

### THOMA-ZEISS HÆMOCYTOMETER

**Thoma-Zeiss Hæmocytometer.**—The instrument now most used for determining the number of red and white cells to the cubic millimeter of blood is the Thoma-Zeiss apparatus.

In consists essentially of a mixing pipette, in which the blood can be diluted to a proper degree, and a counting chamber in which the corpuscles can be allowed to settle on a ruled surface and enumerated by the microscope.

The counting chamber consists of a heavy plate-glass slide on which are cemented two glass plates. The larger of these is square with a central space within which is cemented a smaller circular plateau which is ruled on its upper surface (Fig. 11). The two plates are separated from each other by a narrow circular moat. The inner disk which carries the ruling is exactly one-tenth of a millimeter below the polished upper surface of the outer one. The ruled surface (Fig. 12) is one millimeter square and is divided by a series of fine lines into four hundred smaller squares, each with an area of one four-hundredth of a square mil-

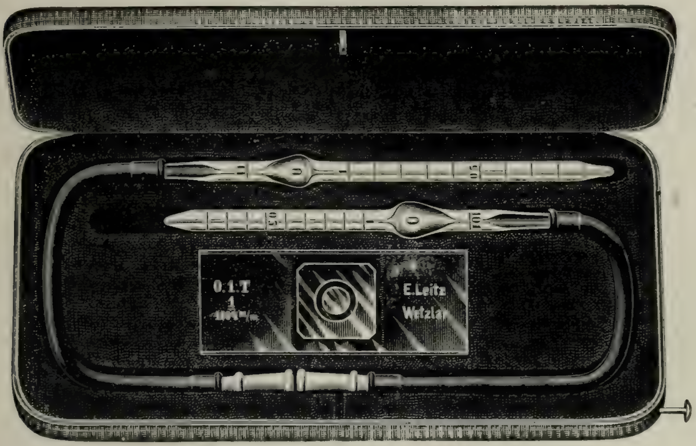

FIG. 10.—THOMA HÆMOCYTOMETER.

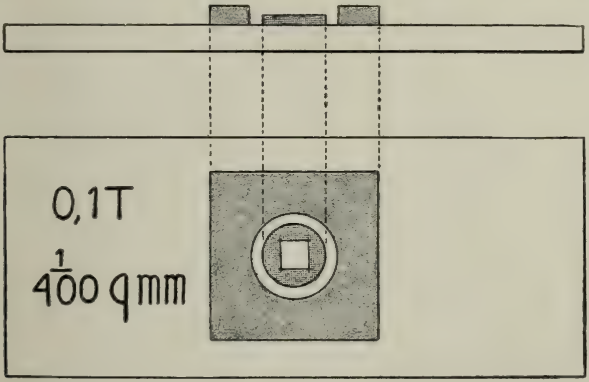

FIG. 11.—DIAGRAM OF THOMA COUNTING CHAMBER.

In the upper figure the difference in level between the plateau and the glass disk surrounding it is purposely exaggerated.

limeter. Every fifth square is accentuated for convenience in counting by being doubly ruled.

The mixing pipette for red cells is a stout glass tube, with a capillary bore at its lower extremity, expanding into a small chamber near its upper end. The capillary portion is so calibrated that its capacity to the mark 1 is just one-hundredth of that of the chamber when filled to the mark 101. The tip of the capillary should be conical and polished so that the presence of a small air bubble in the lower end can be easily noted. The short form of pipette is much more convenient to manipulate and clean, and if care is taken to adjust accurately the column of blood in the capillary, it is quite reliable. The absolute capacity of the instrument varies with each pipette, only the relative dimensions are constant. The capillary portion is also divided into tenths, so that it can be filled with blood to any desired point. An especially accurate form of pipette is the Miescher model made by the firm of Zeiss, in which the marks extend entirely around the tube, thus obviating errors due to parallax. Above and below the marks .5 and 1 are also two extra rings which enable the observer to allow for slight errors in the adjustment of the blood column. If the blood has been accidentally drawn too high and reaches the first mark above 1, the final result can be corrected by adding 1 per cent. to the count without necessitating a tedious adjustment of the blood column. The pipette for counting white cells is exactly similar, except that the capillary is one-tenth the capacity of the chamber.

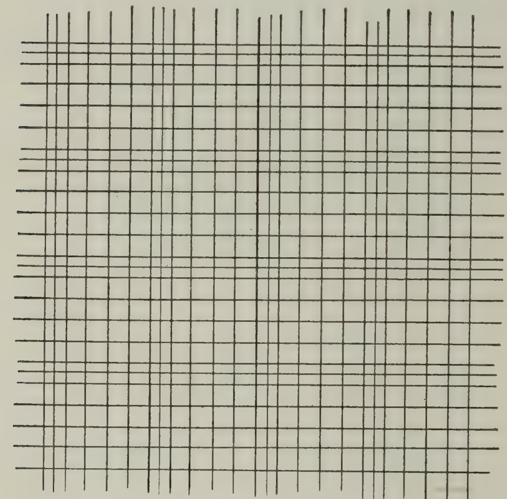

Fig. 12.—Ruling of Thoma-Zeiss Counting Chamber.

There are five stages in the process of counting blood:

1. Obtaining the blood.
2. Diluting and mixing the blood.
3. Filling the counting chamber with the properly diluted blood.
4. Counting the cells.
5. Cleaning the chamber and pipette.

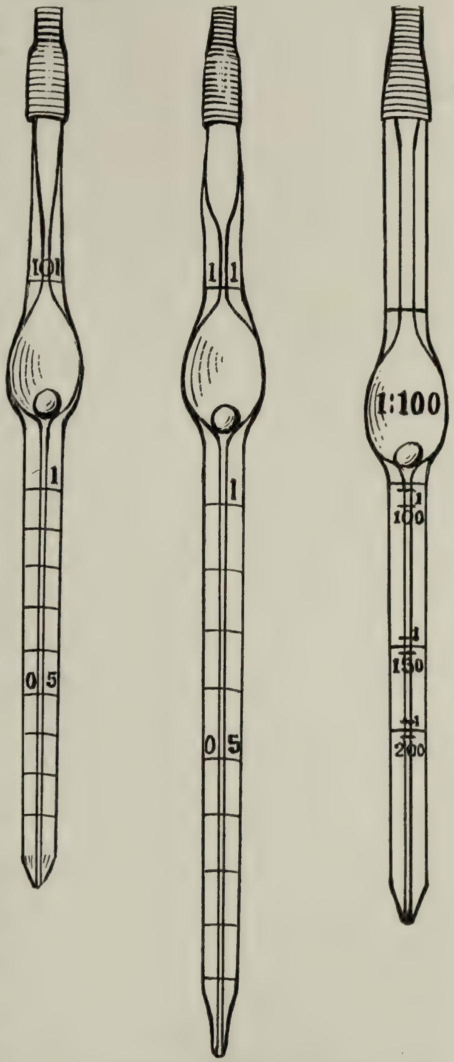

Fig. 13.—Mixing Pipettes for Red and White Corpuscles. Miescher form is on the right.

### COUNTING THE RED CELLS

**Obtaining the Blood.**—The finger is punctured and after a good sized drop has been obtained, without pressure or constriction, the tip of the pipette is steadied by resting on one of the fingers of the hand which holds the patient's, and by gentle suction the capillary bore is filled either to the mark .5 or 1. If the blood is nearly normal, use the mark .5; if anæmic, fill to the mark 1. If the column does not stop exactly at the mark, the tip can be gently wiped with the finger, which will remove a little blood and adjust the fluid to the proper point in the tube. In the pipettes made with extra divisions around the marks .5 and 1, the error can be allowed for in the computation after counting, and no time need be lost in attempting to adjust the column of blood. The importance of this lies in the possibility that if too much time is spent in adjustment the blood will clot in the capillary. In such case the removal of the coagulum may be difficult.

**Diluting the Blood.**—The moment the column is adjusted the tip of the pipette should be cleaned from any adherent blood by wiping with the finger and then dipped into the diluting fluid. The latter should stand ready at hand so that no time is lost. The instant the tip of the pipette is immersed in the diluting fluid, strong suction should be begun to wash the blood up into the pipette and avoid the risk of any clot formation. As the fluid rises slowly in the chamber, the pipette should be carefully rotated to avoid leaving small air bubbles attached to the inner surface of the bulb or the glass mixing bead, and thus vitiate the result. When the fluid reaches the narrow funnel-shaped portion near the mark 101, the suction should be very slight, as otherwise there is danger of the fluid being carried past the mark 101 and the whole result spoiled. The moment the column reaches the mark 101, the tip should be removed from the fluid and the pipette turned horizontally to prevent the fluid from escaping. The finger is then placed tightly over the pointed end, and the whole shaken gently for about a minute. The small glass bead in the chamber facilitates the even distribution of the corpuscles in the fluid.

The count is best made immediately, as the results obtained by carrying the mixture for any length of time in the pocket or in a case are open to serious criticism, especially if the dilution has been made with 3 per cent. sodium chloride, or Toison's solution, instead of Hayem's, which hardens and preserves the corpuscles. If it is impossible to make the count within a few minutes, the best method of carrying the pipette is to remove the rubber tube and mouthpiece and pass a short, stout rubber band over the ends of the pipette, which should then be kept horizontal until used.

*The Diluting Fluids.*—1. Three per cent. sodium chloride solution, colored with gentian or methyl violet until it is opaque in layers thicker than a few centimeters. Sp. gr. 1.022.

2. A solution of sodium sulphate containing 17.6 grams of the dried salt or 40 grams of the crystals of $Na_2SO_4 + 10\ H_2O$, which is isotonic with the red corpuscles and does not alter their size or shape. The specific gravity of this fluid is 1.015, and as it can be colored with gentian violet it is preferable to the 3 per cent. salt which is not isotonic and distorts the red cells. If a

small quantity of "neutral red" is added to the sodium sulphate solution, as suggested by Bettmann,[1] it is possible to distinguish between the nucleated red cells and the leucocytes of the blood in the leukæmias, thus rendering possible an accurate enumeration of both forms of cells at one operation.

A sodium sulphate solution containing 120 grams of the crystallized salt to the liter does not deform the red corpuscles any more than 3 per cent. salt, and as it has a specific gravity of about 1.047 at 18° C., it is much more easy to make an even blood mixture in the counting chamber, as the red corpuscles, whose specific gravity is about 1.088, do not settle out so rapidly as in the other diluting fluids.

3. Toison's Fluid:

| | | |
|---|---|---|
| Methyl violet, 5 B | .025 | gram. |
| Sodium chloride | 1 | " |
| Sodium sulphate | 8 | grams. |
| Glycerin | 30 | c.c. |
| Aq. dest | 160 | c.c. |

4. Hayem's Solution:

| | | |
|---|---|---|
| Mercuric chloride | .5 | gram. |
| Sodium sulphate | 5 | grams. |
| Sodium chloride | 1 | gram. |
| Aq. dest | 200 | c.c. |

Hayem's solution has the disadvantage that it can not be colored with gentian violet. Its special advantage is that it preserves the red cells permanently, and as its specific gravity is about 1.030 the corpuscles settle slowly and the distribution in the counting chamber is a very even one. The use of .9 per cent. physiological NaCl solution is not advisable, as the low specific gravity of this fluid (1.007) allows the cells to settle so rapidly that it is difficult to get an even distribution of the red cells in either the mixing pipette or on the stage of the counting chamber.

**Filling the Counting Chamber.**—Two or three drops are blown out from the pipette in order to remove the diluting fluid which remains in the capillary tube, and to obtain a sample of the well-mixed blood; then a small drop of the latter is placed on the small raised platform in the center of the counting chamber, and immediately, but slowly, the thick cover glass is lowered into place without sliding. If the cover is slid over the drop, part of the latter will be dragged into the moat surrounding the raised platform, or between the cover glass and the upper surface of the outer glass plate, both errors in technique which alter the result of the count. The drop should just cover the whole of the raised platform. When the cover is in place and both it and the plate-glass body of the slide are perfectly clean and free from dust, a very little pressure near the edge of the cover will bring out colored diffraction curves, the so-called Newton's rings. They simply indicate the absolute contact of the two polished surfaces and the perfect adjustment of the instrument, but at times they are difficult to obtain, and are not necessary for an accurate count.

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[1] Münch. med. Woch., 1901, p. 957.

**Counting the Cells.**—After waiting for a minute or so for the corpuscles to settle, the chamber is placed on the microscope stage; and using a No. 5 Leitz, or an 8 mm. Zeiss apochromatic objective, search for the upper left corner of the ruled surface. Begin at the upper left small square and count to the right through five squares; the double ruling every five squares will prevent any error. Then count the five squares in a line just below, and so on until the corpuscles in a block of twenty-five squares have been counted. Repeat until one hundred squares have been counted, not using contiguous blocks of twenty-five, but rather counting along the diagonal of the whole ruled surface, so as to eliminate any error which might arise from slight differences of distribution of the corpuscles on the stage. Some of the cells will lie on the lines. To avoid omitting or counting such cells twice it is necessary to adopt some regular system by which the cells on certain lines shall always be counted in a given square. The simplest is to count all cells to the right and below; that is, any cell lying on a line is counted in the small square lying to the right and below the given line (Fig. 14). The use of a mechanical stage to move the counting chamber is a great aid in the process.

The management of the light is one of the greatest difficulties with beginners. The diaphragm of the Abbe condenser must be reduced to a very small aperture, and it is often better to count by artificial light rather than by daylight, as the amount of illumination can be better regulated.

When the sum of the corpuscles is obtained in one hundred small squares, the number per cubic millimeter can easily be computed from the figures always engraved on the chamber by the maker. (See Fig. 11.) Let us assume that six hundred and sixty-one cells are found in a hundred small squares; then each small square will average 6.61 cells. Now, the chamber is one-tenth of a millimeter deep and each small square $\frac{1}{400}$ of a square millimeter in area;

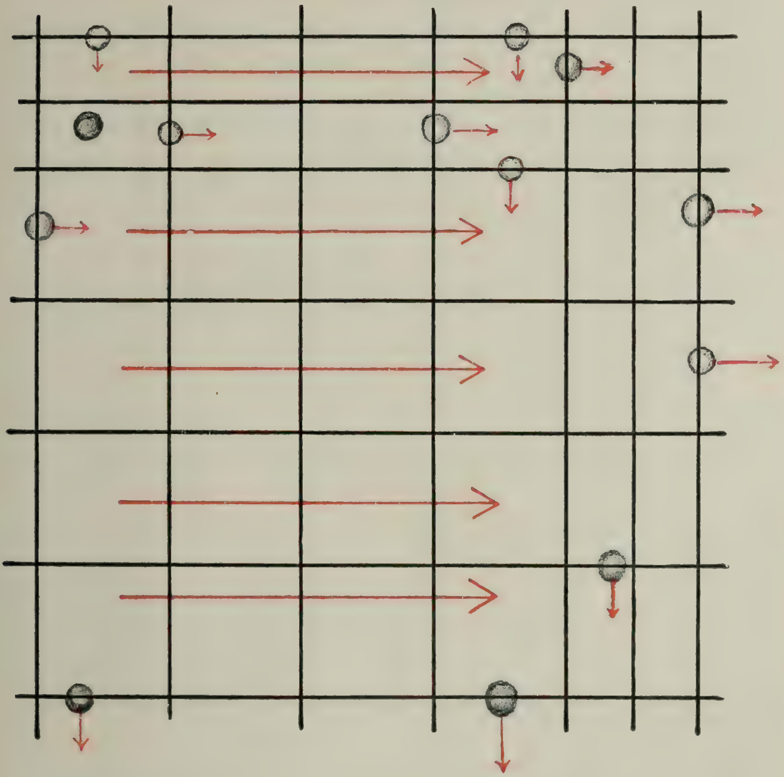

Fig. 14.—Counting Unit of Twenty-five Squares. Arrows show direction of count.

and as the 6.61 cells have settled down from that $\frac{1}{4000}$ of a cubic millimeter overlying the small square, then the number in a cubic millimeter must be 6.61 × 4,000 = 26,440. But the blood was diluted in the mixing pipette one hundred times; therefore the number of corpuscles must be increased one hundred times to give the number in a cubic millimeter of undiluted blood. In other words, 2,644,000 corpuscles are contained in a cubic millimeter of the blood examined.

**Cleaning the Chamber and the Pipette.**—This is a matter of some time and trouble unless either a Bunsen filter pump is used to draw the fluids through the pipette or a throat-spray pressure apparatus is used to blow the cleansing fluids and the air used in the final drying through the tube.

The process should be carried out immediately after using the pipette to prevent the fluids drying in the capillary and blocking it. If any clots are present in the capillary portion, they are best dislodged or perforated by a fine wire such as is employed in cleaning hypodermic needles, or by a stout horsehair. As soon as the clot is permeable, a little strong sodium hydrate solution should be drawn up into the chamber and the whole set aside for an hour to soften and dissolve out the remnant of the clot.

Then wash out with water, alcohol, and finally ether. The pipette is left for a few minutes after the ether has been removed with a gentle current of air passing through it to remove the last traces of the ether vapor and completely dry the inner surface. With a small brass filter pump, which can be attached to the water tap, the entire process of cleaning takes only a few minutes.

Another chemical method which is very useful in removing any coloring matter or small masses of fibrin adhering to the inner surface of the bulb or capillary is as follows: After opening the lumen of the capillary tube with a wire, suck up a little strong alcohol and then remove as much as possible, leaving the walls merely moistened. Then draw in with a *suction pump* a little strong nitric acid. A very violent reaction will take place with the evolution of abundant fumes of $N_2O_4$ and the clots will be rapidly oxidized and removed. Wash with distilled water, alcohol, and finally with ether. The acid should never be sucked up with the mouth, as there is great danger of its being forcibly blown out of the capillary into the mouth or eyes, and when using the suction apparatus, care should be taken never to allow the end of the pipette to point toward the face or clothes of the operator.

Another method which is applicable where Hayem's fluid has not been used, is to digest the clots with pepsin and .4 per cent. HCl. The mixture must be kept in a warm place to act quickly.

The chamber should be cleaned with *water* immediately after using, but never with alcohol or ether, as the cement with which it is mounted is easily softened by these menstrua. It should always be kept in a cool place and out of direct sunlight, as otherwise the cement may become softened by the heat and the depth of the cell be altered.

### COUNTING THE WHITE CELLS

If a very accurate count of the white corpuscles is not necessary, they can be noted at the same time that the red count is made if a 3 per cent. sodium chloride solution tinged with gentian violet, or Toison's fluid is used. The leucocytes then stand out as faint purple cells in contrast to the yellow erythrocytes. The

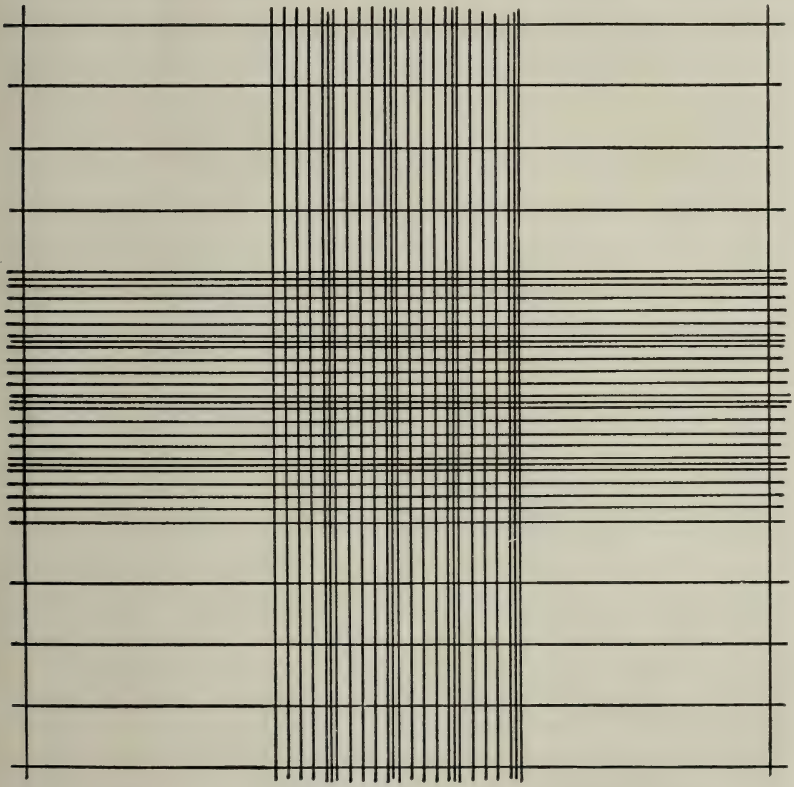

FIG. 15.—RULING OF ZAPPERT-EWING COUNTING CHAMBER.

spaces counted, however, must be much greater than the number gone over for the red cells, and it is best to use one of the special chambers devised by Zappert and modified by Ewing (Fig. 15) in which the central square millimeter of small squares is surrounded by eight other squares of the same size, thus permitting the counting of the leucocytes over an area of nine square millimeters. Breuer[1] has described a useful form of counting cell with a simple ruling which is especially designed for leucocytes.

A far better method, when accuracy is important, is to use a special pipette, diluting the blood one to ten, and to use a 1 per cent. acetic acid mixing fluid, which renders the red cells invisible and brings out the leucocytes very sharply. Some observers recommend coloring the fluid with a small amount of methylene blue or gentian violet, so as to stain the nuclei of the leucocytes, but this is not necessary.

Türk[2] recommends a mixture of the following composition:

| | | |
|---|---|---|
| Glacial acetic acid | 3 | c.c. |
| Distilled water | 300 | " |
| Gentian violet, 1% aqueous solution | 2 to 3 | " |

With a good light and suitable lens it is then possible to distinguish the polynuclear cells from the lymphocytes and thus make a differential and a total count at the same time.

It is also possible to determine the number of eosinophiles in the blood by using a mixture suggested by Zappert.[3] The blood must be diluted one to one hundred instead of one to ten.

The capillary mixing pipette is filled to the mark 1 and the blood washed into the mixing chamber with a sufficient quantity of a 1 per cent. solution of osmic acid to half fill the chamber. The acid is allowed to act on the corpuscles for two minutes and the dilution is then completed to 101 with a mixture containing:

| | | |
|---|---|---|
| Distilled water | 55 | c.c. |
| Glycerin | 45 | " |
| 1% aqueous eosin solution | 17 | " |

In a short time the eosinophiles will be deeply stained and can be separately enumerated during the leucocyte count.

A simpler mixture by which a complete differential count of the leucocytes may be made in the counting chamber, is that of Zollikofer.[1] Two solutions are employed of the following composition and mixed just before using in equal bulks:

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[1] Berl. klin. Woch., 1902, p. 954.

[2] Wien. klin. Woch., 1902, p. 715.

[3] Cent. f. klin. Med., 1892, p. 715.

A.

| | | |
|---|---|---|
| Yellow eosin, soluble in water | .05 | gram. |
| Formalin solution, concentrated | 1 | c.c. |
| Distilled water | 100 | c.c. |

B.

| | | |
|---|---|---|
| Methylene blue, "B X" | .05 | gram. |
| Formalin solution, concentrated | 1 | c.c. |
| Distilled water | 100 | c.c. |

The blood is diluted 1 to 20 with this mixture and the enumeration is carried out after the cells have stood for about five minutes in the counting chamber in order that staining may take place. The red cells have been laked and are not visible.

Onuf recommends a mixture composed of equal parts of 5 per cent. formaldehyde, 1 per cent. sodium chloride solution, a 5 per cent. aqueous solution of eosin, ''w.g.,'' and of Grübler's polychrome methylene blue solution. These four solutions are to be mixed at the time of using and the blood diluted one to one hundred. Using a thin cover and a high power apochromatic dry lens or an oil immersion, it is possible to make a complete differential count of the leucocytes in the chamber, after the preparation has stood for half an hour.

In the writer's opinion it is much better to reserve the counting chamber for simple enumeration of the leucocytes as a whole, and to differentiate the cells in a stained smear rather than to attempt to save time by making a differential chamber count. Abnormal forms are difficult to recognize in the counting chamber, and the granules, with the exception of the eosinophile, are not well demonstrated. Polychromatophilia and granular degeneration of the red cells must always be searched for in smear preparations.

The technique of leucocyte counting is the same as that for the red cells, except that a larger drop of blood is necessary to fill the coarser bore of the capillary portion of the pipette, which is rather more difficult to handle, as the fluid will run out if it is held vertically. It is necessary, therefore, to hold the pipette almost horizontally while drawing up the diluting fluid, and to be very careful not to allow the mixture to run out again when the suction is stopped.

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[1] Zeit. f. wissen. Mik., Bd. xvii, 1900, p. 313.

The white cells can be counted over an area of a square millimeter, which is just included in the field of a No. 3 Leitz lens, and the whole series of nine squares run over in a few minutes. The results are much more accurate than with the one one-hundredth dilution and stained cells. The only source of error is the counting of the nucleated red cells as leucocytes, but it is rare that there are sufficient erythroblasts to alter the result perceptibly.

If the nucleated red cells are very numerous a careful differential count should be made from a stained smear to determine the relative proportion of these cells to the leucocytes. When the relation between the nucleated reds and the leucocytes has been found, a proportional reduction can be made in the white cell count.

The multiplying factors are the depth of the cell, one-tenth of a millimeter, the area counted, one square millimeter, the dilution, ten times. For example, if an average of fifty-six leucocytes were found in each square millimeter, then $10 \times 10 \times 56 = 5{,}600$; in other words, multiply the number found by one hundred. Where the leucocytes are very abundant, as in leukæmia, the blood should be diluted one to twenty or even one to one hundred in a red cell mixing pipette.

### LIMITS OF ACCURACY IN COUNTING BLOOD

The errors which may arise in the enumeration of the red and white cells are of two varieties: the variable and the constant.

The constant errors are those which are due to the inherent defects in the construction of the apparatus. The mixing pipettes frequently show slight errors due to the relation between the capillary and the bulb not being exactly one to one hundred.

Four different pipettes for red cells from two standard makers, showed the following variations: In two of recent make of the short form, the proportions between the capillary to the mark 1 and the bulb, instead of being one to one hundred, were 1 to 101.3 and 1 to 102.7, while a long pipette from another maker showed a relation of 1 to 96.9 and a short pipette, 1 to 114.9. The latter instrument is evidently grossly incorrect, and blood counts made with it first called attention to the defect. Reinert,[1] who examined two pipettes, found an error of under 1 per cent., but a revision of his computations with the use of more accurate values for the specific gravity of water and mercury, shows that the actual error was at least 1 per cent.

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[1] Die Zählung der Blutkörperchen, Leipzig, 1891, p. 44.

**A** second source of error lies in variations in the depth of the counting chamber. The writer has frequently found that the chambers obtained from reliable dealers varied considerably in depth after being exposed to hot summer temperatures, the alteration being due to softening of the cement by which the plateau and the ring surrounding it are fastened to the slide. Exposure to the direct rays of the summer sun will soften the cement in a few minutes.

The variable errors are those resulting from irregular distribution of the cells in the counting chamber and the enumeration of an insufficient number of the corpuscles. For the red cells, the error in counting only one hundred squares is considerable, certainly over 5 per cent. of the total, so that the last four figures as given on page 43 can be omitted and the count considered as approximately 2,640,000. To reduce this error to 3 per cent., it is necessary to count at least four hundred squares, and preferably from several different drops, in order to minimize the error resulting from imperfect mixing of the blood in the pipette.

In enumerating the leucocytes some two hundred cells must be counted to bring the error within 5 per cent. It is necessary, therefore, to make four preparations of a one to one hundred mixture in order to find sufficient leucocytes in a Zappert chamber enclosing nine square millimeters, if the blood be normal. It is evident that a single count made with a 1 to 100 dilution must possess a very large error, and it is therefore preferable to use a one to ten pipette and an acetic acid mixture for dilution.

## INDIRECT METHODS FOR APPROXIMATELY ESTIMATING THE NUMBER OF BLOOD CORPUSCLES

**From Stained Smears.**—Einhorn and Laporte[1] have developed an indirect method which gives fairly accurate results. It is based upon the fact that in a smear made according to Ehrlich on a cover glass the blood spreads out in a layer of fairly constant thickness. Care must be taken to use a small drop of blood; the blood must be spread between the cover glasses without any pressure, and they must be drawn apart carefully so as not to disturb the even distribution of the cells. The preparations are then stained with Jenner's stain. An even field is selected and the red corpuscles in that field counted by means of an eyepiece containing a diaphragm enclosing a ruled square aperture measuring 3 millimeters on a side. The objective should be a Zeiss DD or a No. 5 Leitz, with eyepiece No. 4. The red cells in the field having been counted, the diaphragm is changed to a No. 2 eyepiece, and all the leucocytes counted without changing the field.

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[1] Medical News, vol. lxxx, 1902, p. 741.

This process is repeated until about six square millimeters of smear surface have been counted, which corresponds to about twenty-five fields. The average number of white cells per square millimeter is then computed, and the number found multiplied by four hundred, which gives the number of white cells in a cubic millimeter. The average number of red cells in a square millimeter should then be multiplied by five hundred, which gives the number in a cubic millimeter. The results are naturally only approximate, as they are based upon computations made by the Thoma-Zeiss apparatus and therefore carry the 5 per cent. error of that method plus the error of this approximate enumeration. Therefore the method can never supplant the hæmocytometer for accurate determinations, and its chief advantage lies in the simplicity of the apparatus and the rapidity with which the count can be carried out, as a red and white cell count can be made in fifteen minutes, while at the same time a differential study of the smear is obtained. Smears can be made at the bedside and examined at leisure.

**The Oliver Hæmocytometer.**—This instrument was devised for the rapid determination of the number of red corpuscles in the blood when the number is nearly normal. The writer's experience with the instrument has been fairly satisfactory in testing practically normal blood, while in anæmias the results have been much less accurate than with the Thoma-Zeiss hæmocytometer.[1] The apparatus is, however, simple and easy of application, so that it is useful in the study of variations in the red blood count which occur under physiological conditions, and also as a means of classifying hospital or dispensary cases into those which have anæmia and those which have not, preliminary to a more careful count with the Thoma-Zeiss hæmocytometer.

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[1] Consult for details on this point, *Baumgarten:* Johns Hopkins Hospital Bulletin, 1902, p. 176.

The apparatus consists of a graduated tube flattened on two sides, a blood-measuring capillary pipette, a small candle, and a pipette for the dilution of the mixed blood. The finger is punctured and the small capillary filled with blood. The superfluous blood is wiped off the end of the capillary with the finger and the contents are washed into the tube by a few drops of Hayem's solution from the pipette. Hayem's solution is then added drop by drop until the first appearance of a fine transverse line of light, the earliest indication of which is obtained by turning the tube on its axis, when the line becomes visible first of all on looking through the flattened side. The fluid is then added until the line is just apparent, when the long diameter of the tube is directed toward the flame. The observations must be carried out in a darkened room free from all cross lights. The observer should stand about ten feet from the flame. Diffuse light from the candle should be excluded by holding the tube between the thumb and forefinger so as to make a frame for it. Then the tube is approached very close to the eye. The dilution need not be carried out at the time the blood is obtained as the tube can be corked and examined at leisure. The results are calculated by reading off the level of the fluid on the graduated scale; mark 100 corresponds to five million corpuscles, mark 20 to one million corpuscles to the cubic millimeter. The accuracy, according to Oliver,[1] when working with blood approximately normal and after some experience, is from fifty to one hundred thousand corpuscles to the cubic millimeter. An increased number of leucocytes interferes with the results so that the instrument can not be used in examining blood in cases of high leucocytosis or leukæmia.

### TOTAL SOLIDS AND TOTAL NITROGEN OF THE BLOOD

The total solids of the blood are determined by evaporating to dryness a weighed amount of that fluid contained in a tared weighing bottle, either in an exhausted desiccator over sulphuric acid at room temperature or not above 50° C. The latter method is preferable. The amount of residue is then determined gravimetrically. It amounts to 21 to 22 per cent. of the blood in healthy persons, and may be much lower in chronic anæmias.

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[1] Blood and Blood Pressure, London, 1900.

The total nitrogen is obtained by oxidizing a weighed amount of the blood, according to the Kjeldahl method, which will be given with full details under the subject of the urine. The normal amount of nitrogen is from 3.5 to 3.7 per cent., equivalent to a proteid content of from 21 to 23 per cent.

### REACTION OF THE BLOOD

The reaction of the blood is alkaline to litmus, due to the presence of disodium phosphate and sodium bicarbonate. It is acid to phenolphthalein and neutral to rosolic acid. When alkalinity is expressed in terms of the ionic hypothesis, that is, by the proportion of free hydrogen and hydroxyl ions, the blood is found to be practically a neutral fluid. During diabetic acidosis the hydrogen ions may be larger in amount than the hydroxyl, in other words, the blood may become very slightly acid. These changes, however, are so small as to be determined only by the most refined methods, the alkalinity of normal blood in terms of OH ions being equivalent to a $^{N}/_{10,000,000}$ sodium hydrate solution.

The fact that a considerable quantity of acid may be added to the blood before indicators are influenced is due to the presence of the disodium phosphate which changes into a monosodium phosphate, the varying content of $CO_2$, and the great acid neutralizing qualities of the proteid present; so that the older titration methods using lacmoid give rather the acid capacity of the blood than the true reaction. This acid capacity is given by various observers as from 200 to 500 milligrams of sodium hydrate per 100 c.c. of blood.[1]

**Löwy's Method.**[2]—In this method Löwy uses five c.c. of blood obtained by puncturing a vein, which is mixed with forty-five c.c. of a $\frac{1}{4}$ per cent. ammonium oxalate.

Five c.c. of the laked solution is then titrated with $^{N}/_{25}$ solution of tartaric acid, using lacmoid paper, which has been soaked in concentrated solution of magnesium sulphate, as an indicator.[3] This paper is best obtained from dealers in chemical supplies as it is difficult to make up. The $^{N}/_{25}$ solution of tartaric acid contains 3 grams of the pure acid to a liter, and one c.c. corresponds to .0016 gram of sodium hydrate. The number of c.c. used multiplied by this factor will give the number of milligrams of alkali present in the blood.

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[1] For a description of the methods used in determining the absolute reaction of organic fluids, see *Asher:* Tigerstedt's Handbuch d. physiol. Methodik, i, 2 Abt., 188.

[2] Pflüger's Archiv, 1894, vol. lviii, p. 462.

[3] For a consideration of normal solutions, see Appendix.

**Engel's method**[1] is a modification of the above for clinical purposes. The apparatus is shown in Fig. 16. The blood is obtained in a small pipette, using .05 c.c. and diluting with distilled water to five c.c. A $^{N}/_{75}$ solution of tartaric acid is then run in from a burette and an end reaction is obtained by removing a drop of the fluid and placing it on lacmoid paper. When there is a distinct red line about a drop of blood placed on the reagent paper, the solution has been neutralized. One c.c. of the tartaric acid solution corresponds to .00053 gram of sodium hydrate.

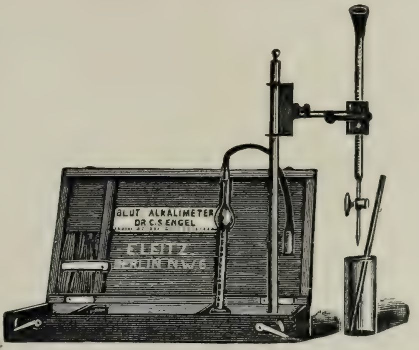

Fig. 16.—Engel's Alkalimeter.

**Dare**[2] has devised a method dependent upon the fact that the two spectral bands of oxyhæmoglobin disappear at the point of exact neutralization of the blood. Twenty c.mm. of blood are placed in a special mixing tube, diluted with distilled water to the zero point, and then a $^{N}/_{200}$ solution of tartaric acid is dropped into the tube, which is inverted after each addition and examined with a spectroscope until the oxyhæmoglobin bands just disappear. The acid solution is prepared by dissolving .075 gram of tartaric acid in distilled water, adding twenty c.c. of 95 per cent. alcohol, and diluting to two hundred c.c. with water.

From a clinical point of view no diagnostic results of value have been obtained from the study of the reaction of the blood.

### COAGULATION TIME OF THE BLOOD

Under normal conditions the coagulation time of the blood is from two to eight minutes, depending a good deal upon the cleanliness of the vessel in which it is received, and upon the amount of tissue juice which is mixed with the blood as it flows from the wound. In disease, prolongation of the time of coagulation has been noted in severe icterus, especially that connected with cirrhosis of the liver, or jaundice due to obstruction of the common bile duct, and also in the blood of persons subject to the hæmorrhagic diathesis (bleeders). The administration of calcium salts shortens the time of coagulation, but after some days the coagulation time returns to the original value. The injection of gelatin causes a shortening of the coagulation time, probably because of the calcium salts constantly present in commercial gelatin.

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[1] Berl. klin. Woch., 1898, p. 308.

[2] Phila. Med. Journal, 1903, p. 137.

The practical value of the coagulation test is chiefly in connection with operations on icteric patients, though the mere fact that the blood coagulates slowly in these cases is not a sufficiently grave symptom to deter from operation when the latter is important.

Another source of error lies in the fact that in some cases of extreme jaundice the blood may coagulate *in vitro* within normal limits, while if the patient be operated upon it may be impossible to check the hæmorrhage and death may result from anæmia. The reason for these differences in the phenomena of coagulation is not as yet definitely known, but it seems to be dependent upon a deficiency in the amount of thrombokinase (see p. 100) given off to the blood by the walls of the blood vessels and by the surrounding tissues. That the slowing of the coagulation is due to the condition of the vessels and tissues rather than to any abnormal composition of the blood is shown by the fact that if the blood of a person suffering from hæmophilia, and the incident slow blood coagulation, be mixed with tissue juice from a healthy person, this blood will then coagulate in a normal time. During a hæmorrhage, also, the coagulation time of the blood from persons suffering from hæmophilia may approximate the normal.

The best method for obtaining the coagulation time of the blood is that of Brodie and Russell.[1] The apparatus employed (Fig. 16*a*) consists of a truncated glass cone (*A*), from whose polished tip the drop of blood is suspended in a chamber. The time of coagulation is determined by gently blowing upon the edge of the drop through a small tube (*B*) in the lateral wall of the chamber in which the drop is suspended. The clips of the microscope stage rest on the projections *C*.

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[1] Jour. of Phys., vol. xxi, 1897, p. 403.

The details of the procedure are as follows: The finger is carefully cleansed and dried. A deep puncture is made so that a free flow is obtained without any pressure. The first drop is wiped off and a sufficient amount of the second is collected so as to cover exactly the tip of the cone, the time being noted at the moment of appearance of the drop. The cone is then inserted in the chamber and the blood drop examined under the low power of the microscope or under a hand lens magnifying thirty diameters. The drop is then blown upon and the corpuscles will be seen to rotate in the drop as shown at *A*. After thirty seconds another puff of air is blown through the tube, and this process is continued until the edge of the drop ceases to spin around and assumes a pasty consistency, and the corpuscles do not move easily. The time should be noted, and the cone promptly removed, and the drop of blood touched to a piece of filter paper. If coagulation has begun the blood will not be completely absorbed by the paper but will leave a clot. The test should be repeated at least once, always using a drop from a fresh puncture.

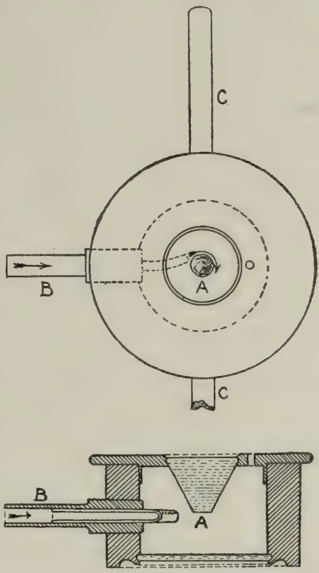

FIG. 16*a*.—BRODIE COAGULATION APPARATUS. The upper figure shows the picture obtained on looking down through the apparatus. The lower figure is a vertical section showing the position of the blood drop and the blowing tube. Natural size.

The clotting of normal blood by this method requires from three to eight minutes, being shorter if the temperature of the air is high, and slower if cold; the average time at a temperature of 20° C. is about five minutes.[1]

## COLOR REACTIONS OF THE BLOOD IN DIABETES

The blood in diabetes shows two reactions which are occasionally of diagnostic value.

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[1] For other details concerning the coagulation time, see *Pratt:* Jour. Med. Research, vol. v, 1903, p. 120. *Blum:* Cent. f. allg. Path., Bd. xv, 1904, p. 385.

**Bremer's Test.**[1]—Rather thick smears of the blood to be tested are made on slides, and also a number of smears of normal blood as a control. All of these smears are then fixed by dry heat by raising the temperature of the oven to 130° C. and then turning off the source of heat. The apparatus is allowed to cool and the slides to be tested and the controls are stained with a freshly made up one per cent. aqueous solution of Congo red. The staining is complete in two minutes and the slides are rinsed in water and dried. The normal blood will be stained a red color; the diabetic blood is either not stained or stained only a faint yellow. The reaction is quite regularly present in cases of diabetes; it is also seen in exophthalmic goiter, neuritis, leukæmia, and rarely in healthy persons. The reaction is given though the sugar may have disappeared from the urine, and hence may be of value in mild cases in which glucose is only occasionally present; also of value in cases of coma suspected of being diabetic, when the urine can not be obtained.

**Williamson's Test.**[2]—Twenty cubic millimeters of the blood to be tested are mixed with forty cubic millimeters of water and one cubic centimeter of a 1 to 6,000 aqueous methylene blue solution. Forty cubic millimeters of liquor potassi (6 per cent. solution) are then added to the contents of the test tube. A control tube of normal blood is made up in the same manner. Both tubes are then heated on a water bath to boiling for four minutes and the change noted. Diabetic blood will decolorize the blue in from two to four minutes, while normal blood causes no change in the color during that time.

## IODOPHILIA

In many pathological conditions, especially in acute infections, a so-called glycogen or iodine reaction or iodophilia may be demonstrated in the bodies of the leucocytes.

The technique is as follows: An unfixed dry blood smear is covered with a solution of the following composition: Iodine, 1 gram; potassium iodide, 3 grams; distilled water, 100 grams; and gum arabic to saturation. A cover glass is pressed down on the fluid and the whole is examined with an oil immersion lens. Two types of reaction can be noted: one is confined to the white cells, the other, a so-called extracellular reaction, is present in the blood plates. The intracellular reaction is the only one of clinical importance. The reaction may occur as a diffuse brown color of the entire protoplasm of the leucocyte, or the protoplasm may contain brown granules which are more or less distinct. In normal blood the protoplasm of the leucocyte takes on a faint yellow, while the nucleus is practically unstained. The red cells show as slightly stained yellowish bodies.

The reaction is supposed to be due to the presence of a compound of glycogen with some albuminous body, though Kaminer[3] claims that it is not dependent upon glycogen. The reaction is

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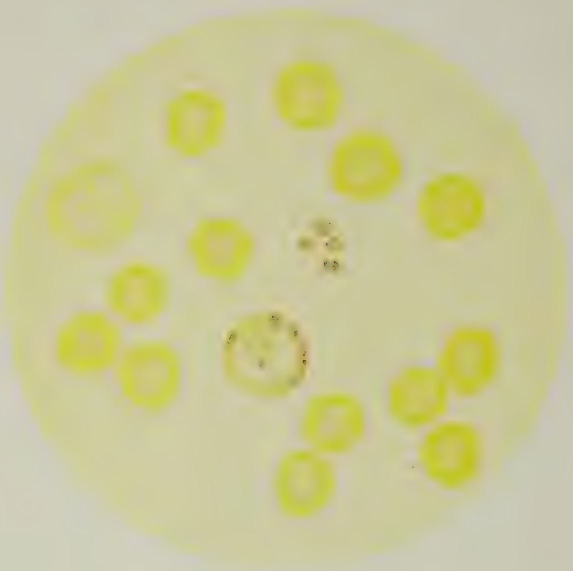
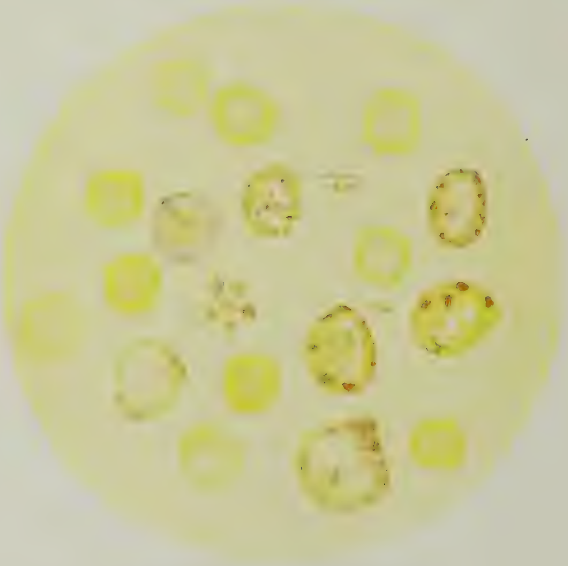
[1] New York Medical Journal, vol. lxiii, 1896, p. 301. [2] British Medical Journal, 1896, Sept., p. 730 (recommends methyl blue); Lancet, 1900, August, p. 320 (recommends methylene blue). [3] Deut. med. Woch., 1899, p. 235.



A.—MODERATE IODOPHILIA.



B.—MARKED IODOPHILIA.

PLATE I.—IODOPHILIA.

# DESCRIPTION OF PLATE I

*Stained with a saturated solution of iodine in gum arabic*

*Figure A.*—IODOPHILIA OF MODERATE DEGREE FROM A CASE OF LOBAR PNEUMONIA

The red cells show as faintly tinged, yellowish, circular bodies without definite structure. The bodies of the white cells take the stain more deeply than do the nuclei. Consequently, the latter appear as lighter areas against the yellowish circle of the cell body. In the leucocytes may be seen a number of brownish granules, scattered chiefly about the periphery. These granules are the so-called glycogen bodies and are not present in the leucocytes of normal blood. Near the center of the field are two small masses of brownish color which are stained blood plates. They are tinged a faint brown color by the iodine. This extracellular glycogen reaction has no clinical importance.

*Figure B.*—VERY ADVANCED IODOPHILIA FROM A CASE OF SEPSIS FOLLOWING APPENDICITIS

The red cells show no change. Some of the leucocytes present contain a considerable number of distinct brownish masses giving the glycogen reaction. Other leucocytes take wholly or in part a homogeneous brown tint. In one cell to the left, near the middle of the drawing, a considerable portion is occupied by a brownish area, the rest of the cell containing numerous fine granules. The blood plates, as usual, show a brownish color due to the presence of glycogen.

quite constant in all cases of sepsis with pus formation, but its intensity is no index of the extent of the suppuration, as it may be no more marked in an extensive general peritonitis than it is in a small abscess of the finger without rise of temperature. It is present quite constantly in pneumonia, malignant disease, and, more rarely, in severe cachexias, and in pulmonary tuberculosis with secondary infection. It is usually but not always absent in acute bronchitis.

The iodophilic granules have been found[1] in pernicious anæmia, secondary anæmia, leukæmia, myxœdema, and in Addison's disease, but not in chlorosis or pseudo-leukæmia. It has been found in the blood of the subjects of chronic morphinism.

The practical value of the reaction is somewhat limited by the large number of diseases which give a positive result. It has been suggested[2] to use the test as a differential between acute articular rheumatism and gonorrhœal arthritis, as it is absent in the former and present in the latter. It is of especial value in appendicitis as an evidence of pus formation when the physical signs, the temperature, pulse, and low leucocyte count do not permit of a positive diagnosis. In a number of cases of this type the writer has seen a marked glycogen reaction, and the presence of a small encapsulated abscess was demonstrated by operation. No reaction was present in a case of acute hæmorrhagic pancreatitis with leucocytosis, which clinically resembled cholecystitis. In uncomplicated cholelithiasis the reaction is not found. No reaction is present in typhoid until after the second week unless some suppurative condition is present.[3]

## DETERMINATION OF THE FREEZING POINT (CRYOSCOPY)

The osmotic pressure which a fluid exerts is due to the number of molecules dissolved in it. These molecules act in much the same manner and under the same laws as the molecules of a gas confined in a given space. Thus the pressure of a gas kept at a constant volume varies directly with the absolute temperature, and this pressure is the same with all gases. In a solution the pressure also varies with the absolute temperature and is not influenced by the nature of the dissolved molecules, but only by their number; that is, all equimolecular solutions have the same osmotic pressure.

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[1] *Hofbauer:* Cent. f. innere Med., 1900, p. 153.

[2] *Sorochowitsch:* Zeit. f. klin. Med., Bd. li, 1903, p. 264.

[3] For further details on this subject see: *Ehrlich:* Die Anaemie, Wien, 1898. *Gabritschefsky:* Arch. f. exp. Path. u. Pharm., 1891, Bd. xxv, p. 272. *Livierato:* Deut. Arch. f. klin. Med., Bd. liii, 1894, p. 303. *Goldberger* and *Weiss:* Wien. klin. Woch., 1897, p. 601. *Best:* Zieglers Beiträge, 1903, Bd. xxxiii, p. 585. *Locke* and *Cabot:* Jour. of Medical Research, 1902, N. S., vol. ii, p. 25. *Locke:* Boston Med. and Surg. Jour., 1902, p. 289.

Solutions which conduct electricity, however, show a greater osmotic pressure than equimolecular solutions of the same substance dissolved in a solvent which does not permit a current to pass. Salts, for instance, when dissolved in absolute alcohol or benzol may not conduct the current, while a marked conductivity is noted as soon as they are dissolved in water. This has been explained by Arrhenius[1] on the assumption that in a conducting fluid the molecules are separated or dissociated into their ions which are capable of carrying electricity. These ions, when free in a solution, act, as far as osmotic pressure is concerned, exactly like molecules, so that solutions of electrolytes have an abnormally high osmotic pressure.

The number of free ions in a given bulk of solution may be determined by obtaining the electrical conductivity of the mixture, while the number of molecules plus ions may be calculated from the depression of the freezing point. The latter is the most convenient indirect method of determining the osmotic pressure of the fluids of the body and their content in molecules, inasmuch as the direct determination of the osmotic pressure by observing the passage of salts through semipermeable membranes offers many practical difficulties.

For practical purposes it is neither necessary to determine the actual number of molecules in a body fluid nor the osmotic pressure which the latter exerts. It is only of importance to obtain relative factors between normal and abnormal fluids. For example, normal blood or blood serum freezes between −.55° C. and −.57° C., while the blood of a person suffering from interstitial nephritis with retention of metabolic residues may freeze at from −.60° C. to −.80° C., or even at 1.04° C.[2] below the point of congelation of distilled water. It is ordinarily sufficient for clinical purposes merely to determine the variations from the normal and not to compute the molecular concentration.[1]

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[1] Zeit. f. physik. Chemie, Bd. i, 1887, p. 631.

[2] *Korányi:* Zeit. f. klin. Med., Bd. xxxiii, 1897, p. 45.

Physiological variations in the osmotic pressure of the blood are slight and transient. The taking of large amounts of fluid or food may cause a variation of a few hundredths of a degree. Starvation lowers the freezing point. *Cyanosis*, due to any cause, lowers the freezing point to a marked degree, and this factor must be always considered in examining blood from any patient in convulsions where insufficient aeration of the blood may occur. The lowered freezing point is due to the increase in the blood of the carbon dioxide molecules. The excess of this gas can be removed by passing a current of oxygen through the blood for a few minutes, or of air, if the treatment be prolonged. The freezing point then rises to the true value due to the content of the blood in molecules derived from salts and organic bodies. Cases of uncompensated heart disease often show a very low freezing point entirely due to the cyanosis and not to retention of metabolic products or nephritis. The blood returns to normal after the compensation of the heart is well established. The blood of eclamptic patients, as a rule, freezes at about −.56° C., but must always be treated with oxygen to remove the $Co_2$. The literature contains a number of reports of lowered $\delta$[2] due to disregard of this source of error.

In the leukæmias and anæmias, the freezing point is practically normal unless a marked lesion of the kidney be present. The writer has seen a $\delta$ of −.57° C. and −.56° C. in pernicious anæmia, and one of −.56° C. in leukæmia. In a secondary anæmia due to carcinoma the $\delta$ was −.60° C., which may possibly have been due to starvation, as the pyloric aperture was completely closed and the patient had had but little food for a week before admission to the hospital.

The freezing-point method, so far as our present knowledge goes, offers no aid in the diagnosis of uncomplicated diseases of the blood. The results obtained in nephritis will be considered under the head of the functional efficiency of the kidneys.

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[1] For further details of the theory and technique of the determination of osmotic pressure by the freezing-point method, see :—*Ostwald-Luther :* Physk.-Chem. Messungen, 1902. *Hamburger :* Osmotischer Druck u. Jonenlehre, Bd. i. 1902. *Jones :* Elements of Physical Chemistry, New York, 1902. *Biltz :* Practical Methods for Determining Molecular Weights, Trans. by *Jones* and *King*, 1898. Traité de Physique Biologique, Tome i, p. 612.

[2] The Greek letter $\delta$ is used to designate the freezing point of the blood, the capital letter $\Delta$ that of the urine.

The freezing point of the blood and other fluids is not greatly altered after death until decomposition sets in. This change occurs earlier in hot weather than in cold, but usually it is not marked for from twenty-four to forty-eight hours. It has been suggested that the results obtained by the determination of the freezing point may be of value in medico-legal cases where it may be of importance to decide between death due to drowning or œdema of the lungs, or to decide whether the body was thrown into the water after death. If death is due to drowning in fresh water, that fluid fills the lungs and dilutes the secretions contained therein so that the fluid expressed from the lungs has a freezing point approaching that of pure water (about $-.25°$ C.). If death has been due to œdema of the lungs the freezing point will be lower, for example, $-.65°$ C., when the alveolar spaces of the organs are filled with water.

Carrara[1] found experimentally that in animals drowned in fresh water the freezing point of the blood obtained from the left side of the heart was lower than that of blood from the right, while in drowning in salt water, the freezing point of the blood from the right heart was lower than that of blood from the left.

Revenstorf[2] reports further investigations on this subject and finds that this condition holds in only a small proportion of the cases so that the method is not very valuable in the determination of death by drowning.

### TECHNIQUE OF CRYOSCOPY

The determination of the freezing point of the body fluids is made preferably by means of an apparatus devised by Beckmann. This consists essentially of a special form of thermometer whose zero can be altered to any point along a scale of five degrees, divided in hundredths of a degree, and a freezing chamber containing the fluid to be investigated, the former being surrounded by an air jacket and the whole inserted into a freezing mixture of ice and salt. The fluid whose freezing point is to be determined is stirred during the process of cooling by a platinum wire which runs through the cork closing the upper end of the freezing chamber.

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[1] Viert. f. ger. Med., 1902, Bd. xxiv, p. 236.

[2] Münch. med. Woch.. 1902, p. 1880. Viert. f. ger. Med., 1903, Bd. xxvi, Suppl. Heft, p. 31. Ibid., 1904, Bd. xxvii, p. 284.

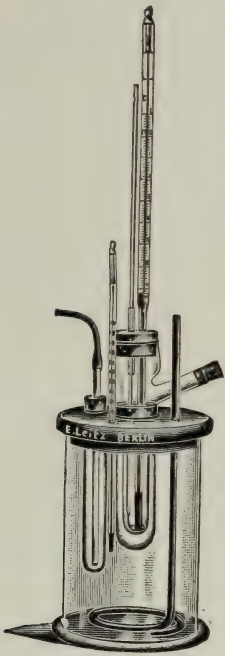

Fig. 17.—Beckmann's Freezing-point Apparatus.

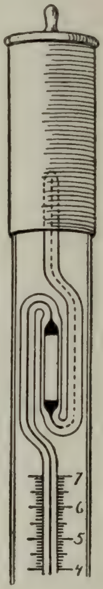

Fig. 18.—Mercury Reservoir of Beckmann Thermometer. Correct Form. (From Biltz.)

A number of modifications of the original model of Beckmann have been devised for clinical purposes. Some forms, as, for example, the Pectoscope,[1] have fixed zero-point thermometers, which, however, offer but little advantage over the original Beckmann form except that they may be handled more roughly without requiring a fresh adjustment of the zero. If, however, the mercury reservoir is of proper shape (see Figs. 18 and 19) the original type is not difficult to work with. The instruments with so-called fixed zero must also have that point determined constantly, as the variations in the glass due to changes in temperature soon alter the zero from the true freezing point. The writer is in the habit of keeping the entire Beckmann apparatus in cold storage when the instrument is not in use. This keeps the mercury always in the reservoir and obviates stress changes in the glass of the bulb. It is not necessary, under these conditions, to redetermine the zero before each set of experiments.

The adjustment of the thermometer is the first operation. The bulb is placed in cracked ice, and after it has been chilled for a few minutes the superfluous mercury in the upper part of the chamber is shaken off by a slight jar of the hand, holding the thermometer about the middle of its length and striking the upper portion against the left index finger (Fig. 20). If, however, the mercury has already fallen to the bottom of the reservoir it is necessary to warm the thermometer with the hand and then invert and jar it (Fig. 21).

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[1] *Zikel:* Klin. Osmologie, Berlin, 1902.

After the inversion, the bulb is cooled with ice which will cause the mercury in the reservoir to join the thread of the mercury in the upper end of the thermometer tube and siphon over as the latter contracts into the bulb. When the thermometer has been filled, and the bulb properly chilled, the surplus should be shaken off as above and then the bulb slightly warmed by lifting it out of the ice mixture. This warming will cause a small particle of mercury to appear at the upper portion of the reservoir. This small globule should be promptly shaken off by jarring the thermometer against the hand and the bulb immediately placed in the ice.

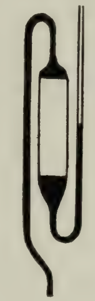

Fig. 19.—Incorrect Form for Reservoir of Beckmann Thermometer, the Upper Portion of the Reservoir Being too Flat.

If after three or four minutes the mercury comes to rest about the middle of the scale the thermometer should be inserted in the freezing chamber, the bulb covered with about ten to fifteen c.c. of previously chilled distilled water and the whole set directly in the battery jar containing the salt and ice mixture. A small aperture in the cover is provided for this purpose. This mixture should be made by breaking ice into small fragments about one to two centimeters in diameter, half filling the jar with these fragments, adding water to within two centimeters of the top of the jar and putting in the proper amount of salt, usually about one hundred grams will suffice.

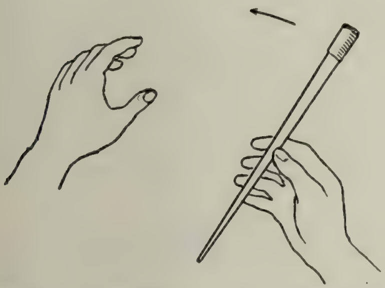

Fig. 20.—Shaking off a Drop of Mercury from Top of Reservoir. (From Biltz.)

The vessel containing the freezing mixture should be covered over with a layer of felt or with a folded cloth so as to diminish the effect of external warmth, and frequently stirred to equalize the temperature of the upper and lower layers, the upper being the colder because of the ice floating on the surface.

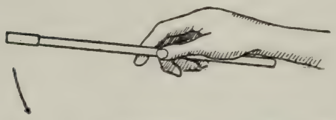

FIG. 21.—SHAKING A DROP OF MERCURY TO THE TOP OF RESERVOIR. (From Biltz.)

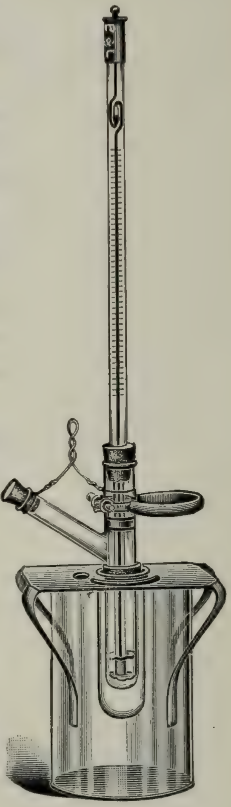

FIG. 22.—IMPROVED MODEL OF BECKMANN FREEZING-POINT APPARATUS WITH MAGNETIC STIRRER.

The distilled water should now be slowly stirred, about one stroke a second being enough, until the temperature of the fluid falls nearly to the bottom of the scale. If the thermometer has been properly adjusted this will be about two or three degrees below the freezing point of the water. If now the distilled water is stirred vigorously, small spicules of ice will make their appearance in the fluid and the thermometer will be noted to rise rapidly at first, then more slowly, until finally it comes to rest. No further stirring will raise or lower the mercury. The instrument should then be read by means of a magnifying glass or a small telescope on an adjustable stand. The readings can easily be made with a hand lens, care being taken that the top of the mercury column, the eye and the scale to be read are all on the same plane, to avoid the errors due to parallax. The figures obtained are the approximate freezing point of water. The ice particles are then melted by warming with the hand and the freezing chamber placed in its air jacket (not directly in the ice and salt as before), and the water is slowly stirred until it is undercooled about half a degree below the freezing point first obtained. It is then "inoculated" by uncorking the side tube and either throwing in a small fragment of ice or touching the stirrer with a fragment and immediately beginning violent agitation of the fluid.

Three or four determinations should be made, using the same distilled water and taking the average of the last three results, as the small amount of air present in distilled water alters the freezing point and the thermometer requires some time to reach its true zero. The air will, however, be removed by the first freezing, so that the true point will be a mean of the later observations or freshly boiled water may be used which contains no air.

The distilled water is now replaced by the fluid to be tested and the same procedure is carried out, or, what is better, the chamber containing the distilled water is replaced by another holding the already cooled fluid. In this way the thermometer is not heated and much time may be saved. If blood is being frozen it need not be defibrinated as the stirring will break up any clot which may form. Several determinations should be made on the fluid and the average taken.

It is almost always necessary to "inoculate" blood in order to avoid excessive undercooling. When the particle of ice falls into the fluid it causes a rapid crystallization in the undercooled blood and the thermometer rises to the freezing point. Inasmuch as the addition of ice somewhat alters the concentration of the blood to be tested, it is better not to allow the ice to fall into the mixture but merely to touch the ring of the platinum stirrer with a small bit of ice, or to "inoculate" with some of the same blood as that being tested. In this case a small amount of the fluid should be placed in a test tube and inserted in the freezing mixture. A glass rod with some absorbent cotton attached to its lower extremity is inserted through a cork or, if preferred, the glass may be drawn out over a flame into a fine thread and then the latter crumpled into an irregular skein while hot.

When the right degree of undercooling has been obtained, the cork in the lateral tube of the freezing chamber is removed and the mass of frozen blood or urine touched to the ring of the platinum stirrer, which is immediately dropped into the undercooled fluid. The undercooled fluid adhering to the stirrer will then freeze, and if the latter is dropped back into the fluid and allowed to strike sharply on the bottom of the freezing chamber, crystallization will usually take place promptly. In cold weather this method is the best, but during the summer months it is almost always necessary to drop a fragment of the frozen fluid into the undercooled fluid in order to start crystallization. The undercooling should not be carried too far, usually not more than half a degree, as otherwise the results will not be accurate.

The stirring can be carried on either by hand or by a mechanical stirring device run by a small motor, or by an electric stirring device in which a ring of iron attached to the wire of the stirrer is regularly attracted and allowed to fall again by the temporary passage of a current through an electro-magnet whose poles encircle the freezing chamber. A metronome or pendulum clock can be arranged to make and break a battery circuit which excites the magnet. When the fluid has been sufficiently undercooled the final determination can be made by the observer. The process usually consumes from twenty minutes to half an hour after the apparatus is set up.

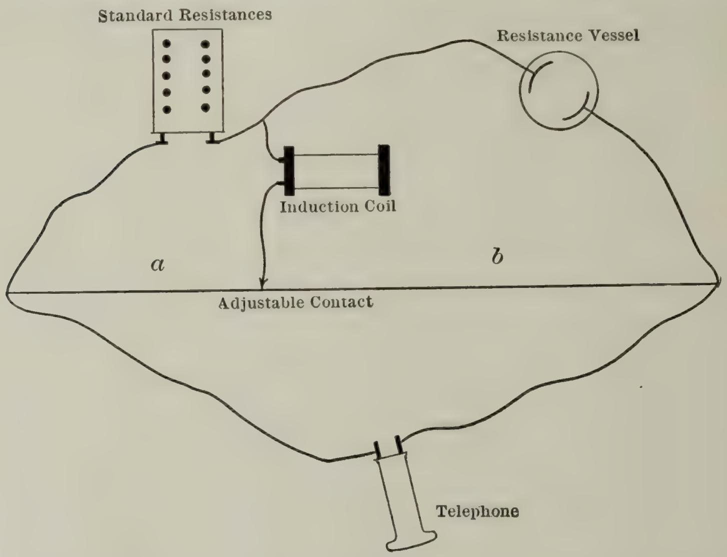

FIG. 23.—DIAGRAM OF WHEATSTONE BRIDGE ARRANGED FOR THE DETERMINATION OF THE CONDUCTIVITY OF FLUIDS.

### DETERMINATION OF THE ELECTRICAL CONDUCTIVITY OF THE BLOOD

The electrical conductivity of a substance is the reciprocal of its specific resistance, and is determined by measurement of the latter. The apparatus used is that ordinarily employed in determining the resistance of wires or other substances, with the exception that instead of using a constant current and a galvanometer, an interrupter and a telephone are used, in order to obviate the passage of a current through the fluid to be tested. This current would cause polarization at the electrodes and chemical alterations in the fluid, and thus render impossible the obtaining of accurate results. The rapidly alternating current of an induction coil causes no such change, but allows of accurate determination of the resistance. An ordinary Wheatstone bridge with a sliding contact and resistance wire may be employed in making the determinations; but it is better to obtain one of the special forms of apparatus devised for this purpose by Kohlrausch and manufactured in Germany, than to use any of the bridges used in wire testing, as the standards employed in the latter are not sufficiently accurate for the purpose.[1]

A diagram of the apparatus is shown in Fig. 23. The fluid to be tested is placed in a small vessel of the form shown in Fig. 24, or more conveniently, of that shown in Fig. 25, which contains a thermometer and enables one to obtain the temperature of the fluid more accurately than the first form, which necessitates the use of a constant temperature bath. The resistance vessel contains two platinum electrodes which have been covered with a layer of spongy platinum deposited by dipping the electrodes in a 3 per cent. watery solution of platinic chloride, to which has been added .025 per cent. of lead acetate. The wires joined to the electrodes are then connected with three or four Daniell cells, or two bichromate cells, and the current allowed to pass until a black precipitate is obtained on the cathode. After about five or ten minutes the current is reversed and the other

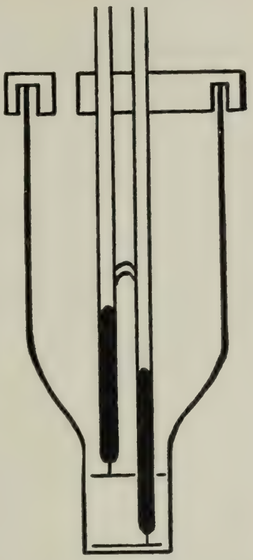

Fig. 24. — Simple Form of Resistance Vessel.

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[1] For full details, see:—*Kohlrausch u. Holborn:* Das Leitvermögen der Elektrolyte, 1898. *Hamburger:* Osmotischer Druck u. Jonenlehre, Bd. i, 1902. *Ostwald-Luther:* Physiko-Chemische Messungen, 1902.

electrode is blackened. The electrodes should then be allowed to stand in distilled water for a long time in order to remove any soluble salts and should be kept in that fluid when not in use.

When a determination is to be made the fluid is placed in the resistance vessel, the two poles of the electrodes are connected with one end of the Wheatstone bridge and with the resistance; the induction coil is then set in action and the sliding contact moved backward and forward on the stretched wire until the point is found where the peculiar humming note of the induction coil can no longer be heard in the telephone, or is just audible. The temperature of the solution should be very carefully determined as the results vary greatly within narrow ranges.

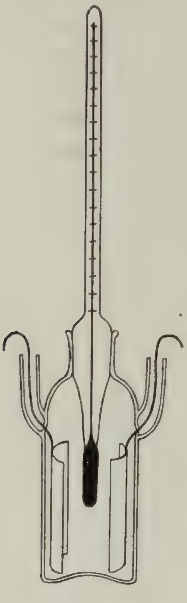

Fig. 25.—Improved Form of Resistance Vessel.

The calculation of the results is as follows: If the resistance of the fluid which is being tested be W, and R is the constant resistance inserted in the rheostat, and a and b the relative lengths of the wire on either side of the sliding point of contact which results in the reduction of the telephone sound to a minimum, then $\frac{W}{R}=\frac{a}{b}$ or $W=R\times\frac{a}{b}$,

The value of R is known from the marks on the resistance box, and a and b being measurements on the wire, W can be found by simple calculation. The value of W, however, depends upon the area of the platinum in the resistance vessel, and therefore the specific capacity of the vessel must be known before absolute results can be obtained. It is therefore necessary to place in this resistance vessel a fluid of a known conductivity and by the determination of the W of the fluid calibrate the vessel. The fluid most convenient for this purpose is a $^{N}/_{10}$ potassium chloride solution of which one liter contains 7.457 grams of the anhydrous chemically pure salt. The specific conductivity of this fluid is .0105 at 15° C. and .0128 at 25° C.

The capacity of the vessel C = specific conductivity × W. The value of W is found by observation to be, for example, 115. The specific conductivity of the standard fluid at 15° C. is .0105

$\times$ 115 = 1.207. The blood or other fluid to be tested is now placed in the calibrated vessel and its resistance W determined at the same temperature, 15° C. The conductivity of the blood will then be $\frac{1.207}{W}$.

For normal human blood serum the equivalent conductivity has been found to be quite constant and about 106. to 119. ($10^{-8}$ ohm $^{-1}$) at 25° C.[1] Whole blood has a much higher resistance due to the presence of the corpuscles which act as non-conductors, and as the results are very variable, investigation has been chiefly confined to the serum.

The study of the conductivity of human blood serum in disease has not led to the discovery of any facts of present practical value; but one observation of considerable scientific interest has been made, which is that in nephritis, even in the uræmic stage, the conductivity of the serum is not sensibly increased and may even be low, though the freezing point is lowered. This is an evidence that in advanced nephritis the chlorides and other substances capable of increasing the conductivity are either not retained in the circulating blood or are in organic combination, thus preventing free action. The lowering of the freezing point is due then to retained organic molecules, chiefly urea, uric acid, purin bases, etc.

## III. THE MORPHOLOGY OF BLOOD

### THE PREPARATION AND EXAMINATION OF FRESH BLOOD

If the blood is to be examined in the fresh condition, the top of a small drop is touched with the center of a cover glass and the latter immediately dropped on a slide, when the blood will spread out in a thin layer between the two, and is ready for examination with the microscope. To obtain perfect preparations, certain precautions are necessary. The cover glass must be very thin and chemically clean; the slide also should be cleaned carefully. The finger tip must be dry, so as not to alter the outflowing blood by admixture of foreign fluids; the drop must be small, so as not to fill up the entire space under the cover glass, as the layer of blood will be too thick under such circumstances. The preparation should consist of only a single layer of cells in the thin central portion, but allow of some rouleau formation near the edge. The cover should not touch the finger when the blood is obtained, for after such an error of technique fat, dirt particles, and epithelial cells are invariably found in the preparation. Any pressure, or sliding the cover glass sideways, will alter the shape of the red cells and spoil the spread. Such fresh preparations can be preserved for some time if carefully ringed with vaselin; filaria will keep active in this way for three to four days at room temperatures.

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[1] *Viola:* Rev. ven. di sci. med., 1901, t. xxxiv, p. 357. *Bickel:* Zeit. f. klin. Med., Bd. xlvii, 1902; also Deut. med. Woch., 1902, p. 501. *Richter:* Charité Annalen, Bd. xxvii, 1903, p. 241. *Engelmann:* Münch. med. Woch., 1903, p. 1778. *Bugarszky u. Tangl:* Pflüger's Arch., Bd. lxxii, 1898, p. 531. *Róth:* Virchow's Arch., Bd. cliv, 1898, p. 466.

The red cells are seen as biconcave disks of a pale greenish-yellow color, the concavity showing as a light central area surrounded by a deeper colored border. The shape of the individual corpuscles is quite uniformly circular in normal blood, while in pathological conditions there may be numerous irregular forms, called poikilocytes, which will later be referred to in detail. In thick preparations of normal blood there is a tendency for the corpuscles to group themselves in long rows, with their concave surfaces in contact. This rouleau formation is not seen in severe anæmias. The diameter of the red cell in normal blood is also subject to only slight variations. Crenations about the edge of the red cell appear as the result of the evaporation of the serum and consequent increase in osmotic tension, causing a flow of water from the cell to the serum and a reduction of the cubic contents of the cell.

The white cells are paler than the red; their nuclei are easily seen, as are the eosinophile and neutrophile granulations, if care is taken to focus the immersion lens very accurately on the cell body. The lymphocytes can be distinguished by their large nuclei, narrow cell bodies, and lack of granules. The eosinophile and neutrophile cells show active amœboid changes; the lymphocytes and large mononuclears do not, though slight motion has been demonstrated by special procedures. The blood plates can be seen usually as centers of radiation for the fibrin network. They are very small, colorless bodies of irregular shape and from two to three micra in diameter. The amount of fibrin formed is of some importance and can be roughly judged from the fresh slide.

Very small, highly refractile bodies measuring not over one micron can be seen in the serum and are especially abundant in smears from the bone marrow. They have been named "blood dust" (hæmoconien) by Müller, and are perhaps extruded granules from the eosinophile and neutrophile cells. They have a rapid dancing (Brownian) motion in the serum and do not appear to be concerned in the formation of the fibrin network.

The plasmodium malariæ can be seen in such fresh preparations with ease if the blood is thinly spread and adult pigmented forms are present; its recognition requires an oil immersion lens.

The embryos of the filaria sanguinis can be seen with a medium power if the light be not too intense. The spirochætes of relapsing fever and trypanosomata may also be seen in fresh blood.

## PREPARATION OF SMEARS OF BLOOD AND OF BLOOD-FORMING ORGANS

**Blood Smears.**—Smears of blood for the study of the morphology of the red and white cells may be made either on slides or cover glasses, each possessing some advantages over the other. Those who advocate cover glasses claim that the smears made on them are less liable to artefacts than those made on slides, and that the smears so made are more easily handled, fixed, stained, etc. From this point of view, however, there are many reasons for dissent. The slides are, in the first place, neither so fragile nor so expensive, nor so difficult to clean as are cover glasses. The making of a satisfactory smear is much more easy in hurried ward or clinic work on slides than on cover glasses. A larger area can be smeared on a slide, some portions of which are sure to be sufficiently thin for the study of the morphology of the red cells, while the thicker parts, where the leucocytes are more abundant, may be used to make a differential count.

In routine blood work the use of cover glasses to protect the finished preparation is unnecessary as the immersion oil may be dropped directly on the stained smear. If for any reason the stain is unsatisfactory the oil can be removed with a few drops of benzol or xylol, and a slide stained, for instance, by the Jenner method, can be restained by the triacid without further fixation. For these reasons many observers who are doing a large amount of clinical blood work prefer to use slides instead of cover glasses.

The preliminary cleaning of the slides may be accomplished by breathing on them and then polishing with a dry towel. This procedure, however, is only satisfactory when the slides have not been previously used. Old or very greasy slides must be cleaned with acids (see Appendix). The use of soap, alcohol, or ether seems simply to spread any grease which may be present on the surface of the glass more widely and thoroughly, and to render it impossible to make a satisfactory smear on such a slide. Cover glasses should always be cleaned with acids.

To make a cover-glass smear two covers are necessary, and preferably of rather large size, so that they can easily be grasped by the corners. Some investigators insist that the covers must never be touched with the fingers, as the moisture present on the latter may alter the smeared corpuscles, and therefore advise handling the covers with forceps only. One of the covers is just touched to the top of the fresh blood drop, then immediately

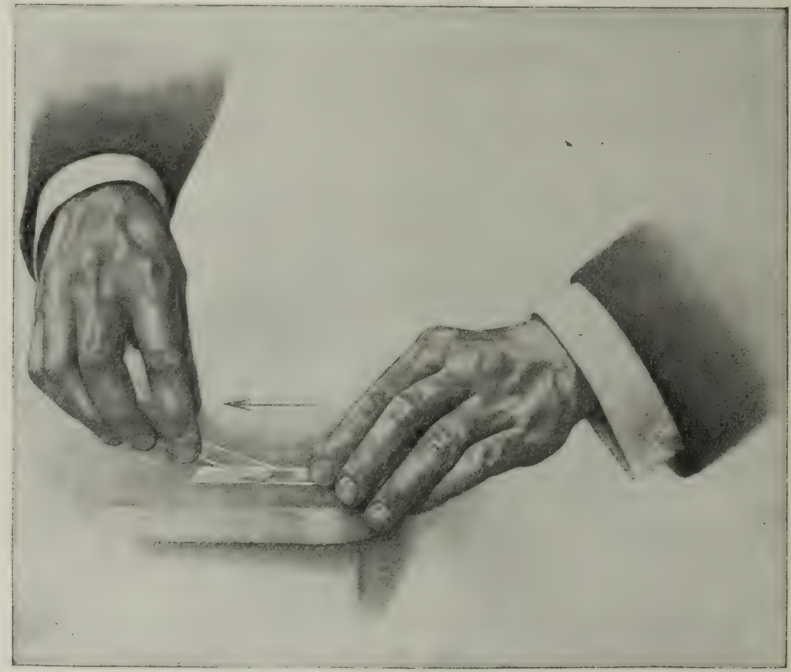

FIG. 26.—MAKING A BLOOD SMEAR ON A SLIDE.

laid flat on the surface of the other cover, so that the drop may instantly spread out between the two in a thin capillary layer. The moment this occurs, the two are to be gently drawn apart by their corners, leaving a thin sheet of blood spread out on each. This should be so thin as to dry almost instantly. Usually one of the covers will be well spread, the other less perfectly. They are then ready for fixation.

A different procedure is used in making smears on slides. The slides to be smeared are laid flat on a table or tray and the drop of blood is just touched with the ground edge of the slide used as a smearer. This smearing slide is then gently touched to the surface of another slide near one end, with the smearing slide at an angle of about thirty degrees to the surface of the slide on which the smear is to be made (Fig. 26). The instant the edge of the slide rests on the surface of the other, the drop of blood spreads out by capillary attraction along that edge. When this occurs, the smearing slide is to be gently *drawn* over the surface of the slide on which the smear is to be made, thus dragging the blood out in a broad film which instantly dries. The size of the drop and the speed with which the smear is made determine the thickness of the preparation and not the pressure, which should always be very light. It is well to vary the speed of the smear during the making, in order that one end may be thick, the other thin.

The writer has found it unnecessary to employ other methods for the making of smears, but some observers have recommended slightly different procedures. Among these may be mentioned one in which a drop of blood is taken up on the edge of a slide and the slide then pushed, edge first, over the surface of another slide, the angle between the two being about thirty degrees. Another method is to smear the blood on the surface of the slide by means of a camel's-hair pencil, or to drag it over the slide by a strip of gutta-percha tissue, or to use a needle to spread the blood. Some of the English hæmatologists have recommended the spreading of a smear by taking up a drop of blood on the edge of a strip of cigarette paper and drawing this over the surface of the slide or cover glass. Blood smearers made of heavy plate glass with a polished end have been placed on the market, but the writer has never been able to obtain as satisfactory smears with any of these methods as with the ground edge of an ordinary slide.

In making differential leucocyte counts on slides, it is well to remember that the white cells are to be found most abundantly along the thick portions of blood left near the end of the spread where the smearer is lifted off the slide. In these situations even in normal blood enough leucocytes can be found in a dozen fields to make a satisfactory count. It is important to know, however, that the distribution of leucocytes in the smear made in this manner is not always exactly the same in the different portions of the preparation. For example, at one end of the smear the lymphocytes may be more numerous than the polynuclears, while at the other end the reverse may be true. The difference is not sufficiently great to lead to error in the ordinary diagnostic study of blood smears; but for scientific purposes it is of advantage to make differential counts of the leucocytes only from very small smears made on cover glasses, the enumeration of the leucocytes to include all the white cells on both covers, in this manner enumerating all the leucocytes in the individual drop of blood used.

**Tissue Smears.**—In making smears of the blood-forming organs the technique is practically the same as that for the blood. Bone marrow is best obtained from the sternal ends of the ribs when there is only a moderate hyperplasia of the marrow or, in advanced anæmias, from the hyperplastic marrow of the shafts of the long bones. It is necessary to press the marrow from the ribs either by means of a bone-cutting forceps or by a vice and to make the smears from the soft jelly-like mass which is extruded. The smears may be made either with the edge of a slide or by dragging some of the marrow over the surface of the slide, or, what is preferable, by touching the slide with the marrow, leaving a series of small areas of marrow tissue. In this way the leucocytes are not so distorted as by the other methods of smearing, but the preparations, as a rule, are much too thick in the center of the drop to permit of satisfactory examination, only the periphery being well preserved.

For the special study of mitotic figures or nuclear structures in the marrow cells, it is necessary to make a thin smear and to harden this smear in some fixing fluid before drying takes place. A good mixture for this purpose is strong ethyl alcohol, to each 100 c.c. of which ten to fifteen drops of strong formalin are added.

After fixation for ten minutes the slides may be stained with hæmatoxylin and eosin, dehydrated with alcohol, cleared with oil of origanum and embedded in balsam without permitting the slide to dry.

The Jenner stain may be used in the following procedure, which the writer has found to give excellent pictures. The slide is removed from the alcohol formalin mixture, washed off in pure methyl alcohol and placed in the Jenner stain. It is left there for three or four minutes, is removed, washed in distilled water to differentiate the structures, and then is dehydrated by carbol xylol or anilin oil xylol, blotting off the excess of water before applying these reagents. The clearing must be rapidly carried out, as otherwise the reagents extract the color from the cells, and the moment the preparation loses its ground-glass appearance it should be washed off with pure xylol in order to remove the carbolic acid or the anilin oil. The preparation is then mounted in acid-free dammar and covered with a cover glass.

Smears from the lymph nodes or spleen are best made by dragging freshly cut portions of one of these organs over the cleaned surface of a glass slide. They may be either dried and stained in the ordinary way or fixed in a moist condition.

### FIXATION OF BLOOD SMEARS

The next step after obtaining proper smears of blood on either slides or cover glasses, is to so fix the corpuscles that they will not be dissolved by the staining fluid, but will take up the dyes in a proper manner. For different stains different methods of fixation are necessary. For instance, after fixation in strong ethyl alcohol the neutrophile granules of the polynuclear leucocytes can be demonstrated only in a very imperfect manner, while after a suitable heating they are easily stained. The classic method, which we owe to Ehrlich, is fixation by heat.

**Heat Fixation.**—The simplest method of heating the smeared slides or covers to the required 110–140 degrees C. is by the use of a long copper plate, about an eighth of an inch thick, heated at one end by a small Bunsen flame or even by an oil lamp or gas burner. After about fifteen minutes, the surface of the plate will have reached a constant temperature, and the site for placing the slides or covers can be determined by dropping water on the surface until a point is found where the water assumes the spheroidal condition. A place about an inch beyond this gives the proper temperature. By beyond is meant away from the lamp.

A more accurate determination can be made by allowing small drops of toluol, B. P. 110 degrees C., or xylol, B. P. 137 degrees C., to fall on the plate and determining the site at which they boil. A temperature of 110 degrees C. for one-half to two minutes is quite sufficient to fix specimens for staining with eosin methylene blue or with the Ehrlich neutrophile stain. It is preferable to place the air-dried smear face down on the hot surface of the copper, as any variations in temperature, due to currents of air in the room, are thus avoided, and also any difference due to the varying thickness of slides or covers.

Small copper ovens are obtainable for the purpose of heating blood slides, and are much preferable to the copper plate, as the temperature can be regulated quite accurately by a thermometer suspended in the center of the hot chamber passing through a perforated cork, which fits in a small opening left for this purpose in the top of the oven. Equally convenient are the ordinary sheet-iron, hot-air sterilizers, such as are used in bacteriological laboratories for sterilizing glassware.

Another useful form of apparatus is what is known to chemists as a Victor Meyer heater. This is a small, flat-topped copper kettle with a long condensing tube, extending vertically, to condense and return to the kettle any of the vapor which may be formed. If a little toluol be boiled in the apparatus, the flat top will immediately assume a temperature of approximately 107° C., while if xylol be used the temperature will rise to about 135° C. There is no danger in either case of the slides being overheated, as there is when a dry heater is used without any special regulating mechanism. The higher temperatures are, as a rule, preferable for blood which is to be stained with the Ehrlich neutrophile stain, as the granules appear much more sharply in preparations that have been carried to about 145° C., which can be obtained by the use of cumol. The red cells, also, under these circumstances, stain of a pale straw color, and the nucleus if present is much better shown than in smears baked at a lower temperature.

A very neat and convenient apparatus for fixing slides has long been used by the writer with excellent results. It is one of the small, flat-topped electric heaters which are sold for domestic use. The temperature produced can be regulated by a rheostat, and at its lowest point it remains quite constantly at from 135° to 137° C. The slides can be faced down on the flat iron top and left until a proper fixation is reached without danger of overheating or injury.

In all cases of heat fixation, it is necessary that the smears be perfectly air-dried before any heat is applied, or the specimens will be ruined. It is equally important not to put the smears in an oven which has not been slightly heated previously, for the vapor from the Bunsen flame, which passes up through the ordinary dry sterilizer, condenses at first on the inner surface of the oven and on any slides which may be in it, and, as the temperature rises, this water will be again evaporated and the slides steamed and ruined. Such preparations show only a faint yellow glaze on the surface where the smear has been, and no trace of any blood structure can be made out by staining. It is therefore always best to allow the temperature to rise to at least 100° C., before the smears are placed in the sterilizer.

**Chemical Fixation.**—1. One of the earliest methods is that of Nikiforoff, by which the slides are placed in a mixture of equal parts of alcohol and ether and left for from fifteen minutes to two hours. The neutrophile granules can be stained by the Ehrlich stain, but the results are not so perfect as by heat fixation.

2. Strong ethyl alcohol for from two to ten minutes. Neutrophile granules are not well shown, but the method is all that is necessary for the eosin-methylene blue stain for parasites of malaria or for filaria. *Methyl* alcohol gives a much better fixation than ethyl, and the neutrophile granules can be stained.

3. Combinations of formaldehyde and alcohol. Benario advises a 1 per cent. solution of commercial formalin in 85 per cent. alcohol; Futcher prefers a $\frac{1}{4}$ per cent. in 95 per cent. alcohol. The fluid is best made up fresh each time. Futcher adds four or five drops of a 10 per cent. aqueous solution of formalin to ten c.c. of 95 per cent. alcohol and uses immediately. The method allows the staining of the neutrophile granules, and is especially good for malaria plasmodia when stained with thionin. The time for fixation is about one minute.[1]

4. An excellent method of fixation is the following: Five cubic

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[1] It should be remembered that when the term formalin is used the commercial solution of formaldehyde is meant.

This solution contains about 40 per cent. of formaldehyde, so that a 10 per cent. solution of formalin is only a 4 per cent. solution of the gaseous formaldehyde.

centimeters of a 1 per cent. solution of osmic acid is mixed with ten drops of glacial acetic acid in a Petri dish. The finger is punctured and the blood smeared on the surface of a slide which has been exposed to the vapor of the osmic acid for two minutes. The slide is then re-exposed to the vapor for one minute. It is then allowed to dry, passed three times through a flame, washed for one minute with a dilute solution of potassium permanganate (a suitable strength has a deep pink color), washed with water, dried, and stained by any of the ordinary methods.[1]

## STAINING BLOOD SMEARS

The methods of staining may be classified as twofold: one aims to bring out a single point in the preparation and to suppress all others; the other is designed to show as much as possible of the various elements present in the smear by staining each a characteristic color. As an example, with malarial parasites thionin and the cell nuclei are the only objects stained. The granules of the mast cells are not abundant enough to interfere, nor will the careful observer mistake granular degeneration of the red cell for small ring parasites. Thus the search for the specific organism is rendered simple, as there is nothing with which it can be reasonably confused. On the one hand, if the preparation were stained with the Jenner, the diffuse terra cotta of the red cells would make the recognition of the plasmodia much more difficult, while, on the other hand, in a preparation of myelogenous leukæmia, the Jenner gives a picture which for wealth of detail and selective staining can not be surpassed.

The theory of the formation of the more complicated of the stains used in blood work is not altogether complete, but an outline is useful in order to understand the technical meanings of some of the terms which have crept into universal use. Stains of the methylene blue-eosin compounds are supposed to be based on the formation, by the combination of these two dyes, of an entirely new substance, which can stain the chromatic substance of the plasmodium malariæ or of bacteria, and yet is quite easily dissociable into its original constituents to seize upon the particular nuclear or cytoplasmic substance for which each dye has some affinity. For example, the blue of the Jenner stains the nuclei, the eosin stains the eosinophile granules, a purple combination of the two colors the neutrophile granules; still another portion of the stain colors the mast-cell granules a purple quite distinct from the nuclear blue of the same mast cell. In order to obtain a constant staining of the chromatic substance of the parasites found in the blood a slightly different combination from that devised by Jenner is necessary, though the latter will occasionally show the chromatic substance of the nucleus in the malarial organism. A more certain form is the mixture devised by Nocht, and improved by Giemsa, in which the eosin is mixed with a neutralized solution containing both methylene blue and methylene azure. The special staining compound is precipitated when the mixture is made, and is only active in this nascent condition and in aqueous solution.

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[1] *Weidenreich:* Folia hæmatologica, vol. iii, 1906, p. 1.

For the explanation of the action of the so-called neutral stains, as suggested by Ehrlich, it is necessary first to define certain characteristics which the different dyes possess. They may be divided into acid and basic groups. The former, represented by orange G. and acid fuchsin, are both disulphonic acid compounds, and give a strong diffuse stain to the cell cytoplasm and to certain specific granules of the leucocytes. Eosin is an acid dye in its general staining action, but belongs to a different class and does not combine well except with methylene blue. The basic dyes are those which have an especial affinity for the chromatic portion of the cell, and are best represented by methylene blue and methyl green. When one of these basic dyes is added to one of the acid type, a precipitate is formed of a new compound, which is capable of staining certain structures not distinctively colored by either constituent. The neutral color is soluble in an excess of the acid dye, and, therefore, if care is taken to have such an excess, there will be formed an aqueous solution of three separate color values—basic, neutral, and acid. Methyl green contains three radicles, which can unite with acid or an acid dye, and in the staining fluid devised by Ehrlich, these three basic radicles are united with the acid dyes, acid fuchsin and orange G., to make the so-called triacid compound with the methyl green. Those portions of the cell which chemically unite with the basic portion of the dye are called basic or basophilic, those which combine with the neutral element are neutrophilic, and those which combine with the acid groups are acidophilic, or, more commonly, eosinophilic, because the reaction was first noted with eosin, which is to be classed with the acid dyes. This affinity for the various portions of the cell has nothing to do with the reaction of the tissues. For instance, the cell bodies of both the lymphocytes and polynuclear cells have an alkaline reaction, yet the first stain with basic dyes, the other with the acid.

**Eosin-Methylene Blue Stain.**—One of the simplest and easiest stains to carry out technically is that by a 1 per cent. eosin solution in 80 per cent. alcohol, followed by a .5 per cent. aqueous methylene blue. If one is staining many slides, the most convenient way is to keep the stains in small jars with ground-glass caps (the so-called Coplin jars are excellent, Fig. 27), and transfer the slides from one to another. The stains will keep perfectly well for months if the ground surface of the cap is smeared with a little vaselin. The eosin to be used is a brand sold by Grübler under the trade name of "Eosin, rein für Blutfarbung, französisch." This will stain deeply enough if used in a .5 per cent. solution; other and less pure kinds need a stronger solution and require more time. Any of the better brands of methylene blue may be used, though the "rectified medicinal" is the best. The eosin is to be used first, for from ten seconds to one minute, depending on the method of fixation and the kind of eosin; the slide is then to be washed in water and dried with blotting paper. The drying may be omitted, but the stain is sharper if it is carried out. It is then dipped in the methylene blue solution for a few seconds to one minute, washed off with water, and dried. The smear should be of a light purple color, not a pure rose pink. Before covering or examining with an immersion lens the slide should be looked over with a low power lens such as Leitz No. 2 or No. 3. The quality of the stain can be judged of quite easily and its depth determined by noticing the amount of color present in the nuclei of the leucocytes. If satisfactory, a drop of immersion oil can be placed directly on the surface of the dry smear and the final examination made with an immersion lens.

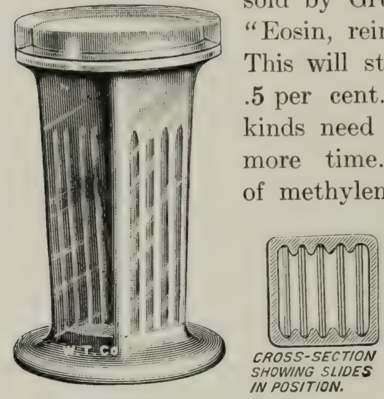
W.T.Co
CROSS-SECTION SHOWING SLIDES IN POSITION.

Fig. 27.—Coplin Jar.

The stain shows the red cells and eosinophile granules of a bright red, the bodies of the polynuclear neutrophiles pink, with the granules shown occasionally when the slide is much overstained with eosin; the nuclei, the mast-cell granules, and the bodies of the lymphocytes are blue.

**Special Stains to Demonstrate the Neutrophile Granules in the Bodies of the Leucocytes.**

(*a*) *The Ehrlich Triacid or Neutrophile Stain.*—This stain has been, perhaps, the most widely used for general blood work, but does not demonstrate the basophilic mast-cell granulations nor satisfactorily stain the plasmodia malariæ, and is being rapidly replaced by combinations of eosin and methylene blue. The smear is preferably fixed by heat, the slide placed in the stain for one to five minutes, washed with distilled water, dried and examined. The nuclei are stained green, the eosinophile and neutrophile granules red; the basophile granules are usually not stained, but occasionally a few granules may take a violet color; the red cells are either red or pale yellow, depending on the temperature of fixation. The higher temperatures give pale yellow corpuscles.

The stain is composed of saturated solutions of methyl green, acid fuchsin, and orange G., in rather variable amounts. Ehrlich's latest formula is:

| | | |
|---|---|---|
| 13–14 | c.c. of | orange G. solution, |
| 6–7 | " " | acid fuchsin solution, |
| 15 | " " | water, |
| 15 | " " | alcohol, |
| 12.5 | " " | methyl green solution, |
| 10 | " " | alcohol, |
| 10 | " " | glycerin. |

The colors used must be chemically pure and added in the order given. It is far more satisfactory to purchase the stain ready made from Grübler than to attempt to make it up. The solution is the best form; that which is sold in powder is less useful, and the mixture known as Ehrlich-Biondi-Heidenhain solution is not suitable for blood work. Pappenheim has modified the stain by substituting methylene blue for the methyl green, which is said to be an improvement, but the writer has had no experience with the new dye.

(*b*) *The Jenner Stain.*—This stain is especially useful in clinical work, as it rapidly fixes and stains the blood at the same time. Its composition is based upon the fact that solutions of eosin and methylene blue mutually precipitate each other, and that the new compound so formed has different staining qualities from the two dyes which go to form it. This eosinate of methylene blue had been made use of by Romanowski to give a differential stain between the body of the malarial parasite and its nucleus, and Jenner[1] modified the method so as to render it applicable to general blood work.

Equal quantities of a 1.2 per cent. solution in distilled water of Grübler's water-soluble eosin, yellow shade, and a 1 per cent. aqueous solution of Grübler's medicinal methylene blue are mixed together in a beaker, well stirred and allowed to stand for twenty-four hours. The metallic looking precipitate is filtered off, washed thoroughly with distilled water until the filtrate shows only a faint blue, scraped from the paper and dried in the air without heating. For use, dissolve half a gram of the dry powder in one hundred c.c. of pure methyl alcohol.[2] The stain may also be made by simply dissolving the eosin and methylene blue in the proper proportions in the methyl alcohol.

Blood smears are made in the usual way on slides or covers which have been cleansed with special care, so that there is no free acid or alkali on them, a point which is essential to proper working of the stain. The slides may be kept in alcohol and dried off when needed, though fresh slides need only polishing with a cloth to render them clean enough. The air-dried blood smears are dropped into the stain without previous fixation and left for from one to three minutes. The specimen is at once rinsed in distilled water till the film has a pale pink color, which usually appears in five to ten seconds. Dry in the air and mount in acid-free dammar. The red cells are stained a terra-cotta color, the nuclei are blue, blood plates purple, the neutrophile and eosinophile granules are red, the basophile granules and cell bodies, bacteria, filaria, and malaria plasmodia, blue. The granules of the mast cells often show a distinct purple shade in contrast to the pure blue of the nuclei of the leucocytes.

Smears which have been kept for a few weeks do not stain as well with the Jenner as do perfectly fresh preparations; as there is a tendency to overstain with the methylene blue component of the mixture. This difficulty can, to a certain extent, be remedied by rinsing the stain as usual in water until no more color is given off, and then extracting the surplus methylene blue by dipping the slide, for a few seconds only, in from 60 to 80 per cent. ethyl alcohol and again rinsing in water. In smears not over a few months old, this process often serves to make excellent preparations, the granulations of the various cells showing as well as in perfectly fresh smears; but in slides which have been kept for a year or more it is difficult to regain the peculiar selective properties which this stain shows in fresh slides, the methylene blue staining everything diffusely. For this reason it is advisable, in storing slides from a rare case for future study, to coat each side with melted paraffin and thus protect it from the chemical action of the air. The paraffin is easily removed by xylol.

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[1] Lancet, 1899, vol. i, p. 370.

[2] Kahlbaum's pure methyl alcohol is expensive, but is the best. Merck's pure alcohol, however, may be used.

**Special Stains for Malarial Parasites.**—(*a*) *The Nocht*[1] *Stain for the Chromatin of the Malarial Parasite.*—The ordinary eosin and methylene blue stain, or the thionin stain, as commonly used in staining the malarial parasite, gives little or no evidence of a nucleus. Occasionally, in a well-stained Jenner preparation, the nuclear chromatin is stained red, but this result is quite inconstant. In order to obtain a nuclear stain for the purpose of investigating the nuclear transformations, Nocht has modified the stain originally devised for this purpose by Romanowski, and has rendered the obtaining of constant results a matter of some ease. He employs aqueous eosin "w.g." and polychrome methylene blue. The latter is a 1 per cent. methylene blue solution which has been altered chemically by heating with .5 per cent. sodium carbonate for two days to a temperature of 55° C. This altered solution contains a dye which when combined with eosin is capable of staining the nuclear substance of the malarial parasite a bright-red color.

The staining mixture is made up by adding two to three drops of a 1 per cent. aqueous eosin to two c.c. of water. The polychrome solution is then added drop by drop until the red color of the eosin is only faintly visible. The preparation, which has been fixed in methyl or ethyl alcohol, is then laid face down in the solution. This is to prevent the precipitate, which immediately forms in the eosin-methylene blue solution, from being deposited on the preparation. Staining is complete in five to ten minutes. Wash in water, dry, and examine with an oil immersion.

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[1] Cent. f. Bakt., I Abt., Bd. xxv, 1899, p. 764.

(*b*) *The Wright Chromatin Stain.*[1]—The stain is a modification of that published by Leishman[2] and is especially suited to the demonstration of malarial parasites and degenerations in the red cells. It is not equal to the Jenner stain for general clinical work.

Preparation of the Staining Fluid.—A ½ per cent. solution of sodium bicarbonate is made up in distilled water, and 1 per cent. by weight of powdered methylene blue is added. Any of the methylene blues sold by Grübler may be used, such as "BX," "Koch's," or "Ehrlich's Rectified." The mixture is then put in a flask and steamed in an Arnold steam-sterilizer for an hour, counting from the time when the temperature of the apparatus is about 100° C. The mixture is then removed from the sterilizer and allowed to cool. When cool it is poured into a dish or flask and a one part to one thousand solution of eosin (Grübler, yellowish, soluble in water) is added, until the mixture loses its blue color and becomes purple with a yellowish metallic scum on the surface. Close inspection shows a fine granular black precipitate in the fluid to produce which requires about five hundred c.c. of eosin solution for one hundred of the alkaline methylene blue. The precipitate is collected on a filter and allowed to dry without washing. When thoroughly dry a saturated solution in pure methyl alcohol is made. Three decigrams of the dry precipitate will saturate one hundred c.c. of alcohol in a few minutes. The alcoholic solution is then filtered, and to eighty c.c. of the filtrate, twenty c.c. of methyl alcohol are added. The object of diluting the saturated solution is to prevent precipitation of the dye on the blood film in the process of staining. This somewhat diluted alcoholic solution of the precipitate is the staining fluid. It is permanent, but should be carefully protected from evaporation and moisture by tight corking.

Staining the Blood Film.—If the blood is spread on a cover glass, the solution may be put in a small Stender or Petri dish; if on a slide, a Coplin jar is the most convenient. The staining fluid is to remain in contact with the film for one minute. The slides or covers are then removed and water dropped on the surface of the smear until a metallic scum forms on the surface of the mixture, and a reddish tint develops at the margins, more dye being added if necessary. The preparation is then left for three to ten minutes, during which time the differential staining takes place. It is then washed in water. The film now has purple or blue color, and if examined with a microscope the red corpuscles will be seen to have a blue stain. The next step is to develop the differential staining of the various forms in the preparation. This is done by washing in distilled water until the thin portions of the smear appear yellowish or a faint pink. If the process of differentiation is to be accurately observed, the slide, still covered with water, can be placed on the stage of a microscope and examined with a low power. The red corpuscles should be pink, the nuclei of the leucocytes, red or purple, the cell bodies of the lymphocytes, a bright blue. As soon as this stage is reached, the slides should be dried off with thick blotting paper, preferably the smooth-surfaced variety used by photographers, called "Royal," covered directly with oil and mounted in xylol dammar. Films of blood which are over one month old do not give good results. The staining process should be longer than as given above, and the washing in water shorter if an intense chromatin stain is desired.

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[1] Journal of Medical Research, vol. ii, 1902, p. 138.

[2] British Medical Journal, 1901, p. 635.

(*c*) *The Goldhorn Stain.*—A useful modification of the Nocht stain has been devised by Goldhorn,[1] by which the blood is fixed and stained by a single operation. The unfixed preparation is covered with the dye. This is allowed to remain on the slide for three or four seconds; the excess of dye is poured off; and the slide is immediately inverted and dipped slowly into water, either tap or distilled. The slide is held in the water for three or four seconds and is then moved about to remove the excess of stain. It is then blotted off with "Royal" blotting paper or set on edge to dry in the air. If a deep stain of the Spirochæte pallida is desired the preparation may be flooded for fifteen to twenty seconds with Gram's solution, which changes the violet tint of the pallida to a bluish black. It is better to obtain the fresh dye through a dealer than to attempt to make up the rather complicated mixture.

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[1] Jour. Exp. Med., vol. viii, 1906, p. 451.

The results are difficult to secure unless a fresh stock of the dye is employed, and when successful are not so clean-cut as those obtained by the Nocht method, though the rapidity of the procedure renders it a useful one for clinical purposes.

The preparation should be examined by a medium power dry lens, and if the nuclei of the leucocytes are of a carmine red the stain is successful and further investigations of the smear may be made with an immersion lens.

(*d*) *Eosin and Methylene Azure Stain.*—Giemsa[1] has shown that these complicated methods of preparation of the dyes for staining malarial parasites can be simplified by the substitution of the pure staining substance extracted from the polychrome methylene blue, the peculiar staining properties of this substance being due to the presence of methylene azure.

This dye can be obtained in two forms from Grübler. One is pure methylene azure, which contains no methylene blue, so that the stain produced is almost wholly a chromatin stain; the second preparation is a combination of equal parts of methylene azure and medicinal methylene blue (Höchst), and is sold under the trade name of "Azure II for blood staining."

The most convenient combination in which to use these dyes is that recently published by Giemsa,[2] which contains Azur II-Eosin, 3 grams; Azur II, 0.8 gram; Merck's chemically pure glycerin, 250 grams; Kahlbaum's methyl alcohol I, 250 grams. It is better to purchase this solution from Grübler or his agents as it is not easy to make. The staining mixture is prepared by adding one drop of the above solution to 1 c.c. of water. The blood slide or cover glass, previously hardened in strong methyl or ethyl alcohol for five minutes, is covered with the diluted staining solution, or allowed to stand in a Coplin jar filled with the same, for ten to fifteen minutes. When an especially deep stain is required, as for instance in demonstrating the Spirochæte pallida, one to two drops of a $\frac{1}{10}$ per cent. solution of potassium carbonate may be added to each c.c. of distilled water. In fifteen minutes the spirochæte is stained with sufficient intensity to be recognized, but it is better to permit the dye to act for one hour as the organisms are then much more readily found. The smear is washed for from five to ten seconds by means of a strong stream of water and mounted, after drying in the air, in xylol dammar or in acid-free Canada balsam. The commercial balsam and dammar, made up with oil of cedar as a solvent, bleach these preparations very rapidly. The smears should not be dried by heat as the stains are faded by a comparatively low temperature, nor should they be allowed to lie exposed to strong light for any length of time, and should be especially protected from direct sunlight, as the characteristic chromatin stain fades in a few hours when exposed to sunlight. As a rule no precipitate is produced in this staining mixture. If, however, on long staining, there is a slight granular deposit on the smear, it can be removed by dipping the preparation in strong alcohol for a fraction of a second and immediately washing off in distilled water. Old preparations should be treated with a more dilute mixture than the above and allowed to stand for a long period, even overnight.

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[1] Cent. f. Bakt., I. Abt. Orig., Bd. xxxii, 1902, p. 307.

[2] *Ibid.*, Bd. xxxvii, 1904, p. 308.

The writer has shown[1] that this process can be simplified for clinical purposes by using stronger solutions of the dyes. The slides should be fixed in strong methyl alcohol for from one to five minutes and then stained with a one to one thousand aqueous eosin solution until the smear assumes a faint pink. The excess of the eosin is then poured off and a few drops of a $\frac{1}{4}$ per cent. aqueous solution of methylene Azure II placed on the surface of the blood.

The staining can be watched under the low power of a microscope, and when the nuclei have assumed a red or purple color the process is completed. Half a minute is often sufficient with fresh preparations. The slide is washed off by a strong stream of distilled water, blotted with smooth surfaced "Royal" paper and dried in the air. If the preparation is over a month old the stain is likely to contain an excess of blue. This can be removed by dipping the slide momentarily into 70 per cent. ethyl alcohol, and washing off with distilled water. One treatment of this sort will not decolorize the chromatin of the malarial parasite. If haste in preparation is not essential, decolorization can also be obtained by allowing the slides to stand in distilled water for from one to two hours, when a more gradual and perfect differentiation of the various forms will take place. One drawback to all these chromatin stains is that, as a rule, the neutrophile granules of the polynuclear cells are not well defined. To obtain a good granulation stain it is necessary to fix the preparations somewhat longer in methyl alcohol, and then to stain them either for a very long time in dilute solutions or for a short time in a concentrated mixture, preferably allowing some of the eosin to remain on the surface of the slide and mix with the methylene azure. As the combination of the two substances is the staining agent, if the slide be washed free from excess of eosin, only that amount of this substance which remains in the cells themselves gives rise to the chromatin stain when the azure is added. It is necessary, therefore, to use a mixture of both stains to obtain deeply colored preparations.

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[1] Medical News, vol. lxxxiii, 1903, p. 248.

(*e*) *Thionin Stain for Malarial Parasites.*—This stain, which was first suggested by Marchoux,[1] is especially useful in connection with smears fixed by formalin and alcohol. It is made by adding to one hundred c. c. of 2 per cent. carbolic acid, twenty c. c. of saturated solution of thionin in 50 per cent. alcohol.

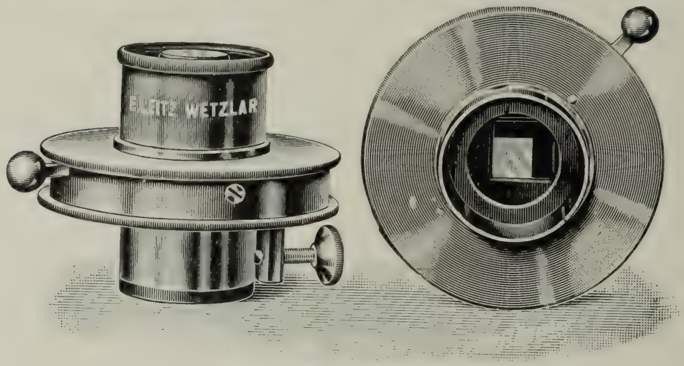

FIG. 28.—EHRLICH EYEPIECE WITH IRIS DIAPHRAGM.

The stain should ripen for a few days before being used, and improves with age. It requires about fifteen seconds for its action. The plasmodia are exceedingly well shown as deep purple, irregular masses in the faint green bodies of the red cells, and their recognition is much easier than in preparations stained either with eosin-methylene blue or Jenner. This is also a good stain for the very thick smears recommended by Ross in searching for crescents, when the number present in the blood is very small. Preparations stained with thionin are usually not permanent, but fade in the course of a year or so even if kept in a dark place, and much more rapidly in the light.

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[1] Ann. de l'Institut Pasteur, p. 640, 1897.

## THE EXAMINATION OF STAINED SMEARS

### DIFFERENTIAL COUNTING

In the examination of stained smears a mechanical stage is of great assistance in thoroughly and systematically searching the preparation, especially when looking for malarial parasites. An oil immersion lens should be used, though when merely looking for megaloblasts in slides from suspected pernicious anæmia, a Zeiss or Spencer 8 mm. apochromatic lens with a high eyepiece is to be preferred. In making differential leucocyte counts an Ehrlich eyepiece (Fig. 28), is very convenient, the field being square and limited to that portion sharply defined by the objective. In making differential counts of the leucocytes in a stained smear, it is best to count at least two hundred for a rough estimate, and five hundred if the results are to be fairly accurate. The results may be tabulated in percentages; thus, polynuclears, 74 per cent., lymphocytes, 24 per cent., etc. Or the proportions obtained may be expressed in numbers per cubic millimeter. Thus, if the total leucocyte count be 10,000 to the cubic millimeter, and there are 70 per cent. polynuclear cells, it is evident that there are 7,000 of the latter to the cubic millimeter. By expressing the amounts in this way it is easy to determine at a glance a relative increase from an absolute increase in the number of any group of leucocytes. Degenerated cells should also be included in the count, especially in the leukæmias.

If large numbers of nucleated red cells are present, they may be enumerated separately and their number to the cubic millimeter determined by comparing the number obtained during the differential count with the number of leucocytes seen at the same time. As the nucleated red cells are usually counted as leucocytes in the hæmocytometer chamber, and if ten are enumerated during a differential count of 490 leucocytes, evidently 2 per cent. of the total number of leucocytes enumerated in the counting chamber are nucleated red cells. If the leucocytosis as determined in the counting chamber was 10,000, and 2 per cent. of that number of cells were nucleated reds, the erythroblasts are two hundred to the cubic millimeter.

## MORPHOLOGY OF THE RED CELLS

### TYPES OF RED CELLS (ERYTHROCYTES) FOUND IN NORMAL BLOOD

In stained smears of normal blood, the cells appear as circular disks with a central pale area, gradually shading off to a deeper color at the periphery. These cells average 7.5 micra; yet even in normal blood there are considerable variations in size, ranging in adults between 6.5 and 9.3 micra, and 3.3 and 10.5 micra in children. With all ordinary stains the stroma of the red cell remains unstained, the hæmoglobin only taking the color. If, therefore, one is familiar with the depth of color which a given stain—for instance, eosin—affords after a certain fixation, then it is possible to judge, with some reserve, of the quantity of hæmoglobin present in the corpuscles in a smear. The variation is especially well seen in cells whose central depressions are well marked, leaving only a faint peripheral ring of stained substance. The normal red cell is orthochromatic; that is, it stains with acid dyes, for example, eosin, and does not take up any portion of the color from the basic group.

In chlorotic, post-hæmorrhagic and secondary anæmias, the quantity of hæmoglobin is usually diminished in the cells quite uniformly, while in pernicious anæmia the quantity of hæmoglobin is normal or relatively increased in the cells; but this does not hold absolutely in every erythrocyte, for even in chlorosis, the type of disease in which the hæmoglobin is reduced, an occasional cell may be found staining deeply and evenly, while in pernicious anæmia ring-shaped erythrocytes may be seen which are only faint shadows of the ordinary cell. The central pale depression varies a great deal in individual normal corpuscles; some have larger, others smaller central depressions; while occasionally a cell stains uniformly with no evidence of the normal biconcavity. This is especially true in smears which have been spread very thin and have dried instantly,.as the corpuscle is fixed in its flattened position by the drying of the film before it can recover its biconcave shape. In the thicker portions of the slide, however, where the extra quantity of serum has prevented instant drying, the central pale area is much more marked. This fact must be borne in mind while examining slides of chlorosis, for in the thin parts of the smear no central depression may be noted in red cells which are highly anæmic. The circular form of the normal red cell is very constant, but occasionally forms are seen a little oval in their outline or slightly pear-shaped (Fig. 29). Care must be taken not to confuse these oval shapes with those seen in roughly prepared slides. When the deformity has been produced mechanically, a large number of cells are affected, and the long axes of the ovals or the tips of the pear-shaped forms all point in approximately the same direction.

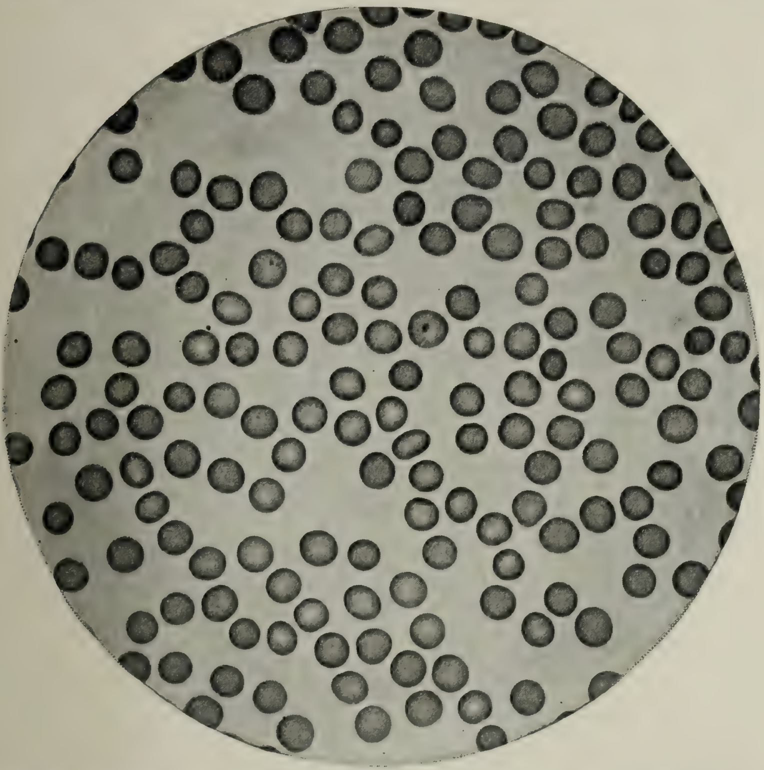

Fig. 29.—Normal Blood. Note the slight variations in size, shape, and depth of coloring. Magnified 700 diameters.

Crenation of the red cells is a very commonly observed artefact, due to the slow drying of the blood smear or to the evaporation of the watery constituents of the serum in fresh preparations. It is occasionally observed in severe anæmias. Crenation is often induced more rapidly and easily than in normal blood, in the blood of persons suffering from acute infections and from chronic diseases, especially those in which there is marked alteration in the composition of the blood, as, for example, in nephritis, acute yellow atrophy, icterus neonatorum, and cirrhosis.[1]

## TYPES OF RED CELLS FOUND IN ABNORMAL BLOOD

### *A. Cells without Nuclei, but of Abnormal Size and Shape*

**Megalocytes** are large cells of regular shape and even staining, generally without a well-marked central depression. They appear in pernicious and severe anæmias. Rarely one sees, in extreme cases, enormous, very faintly stained cells, whose surface shows distinct folds or wrinkles, and whose edges fade away quite gradually into the general pink stain of the serum on the slide (Fig. 30). These dropsical and imperfect cells often show faint remnants of a nuclear structure.[2] Megalocytes may be polychromatophilic or show granular degeneration.

**Microcytes** are very small cells, not over 5 micra in diameter, which are seen in all severe anæmias. They stain deeply and evenly, as a rule, and may show polychromatophilia. (Plate II, Fig. 14.)

**Poikilocytes** are irregular pear or hour-glass shaped cells, which may appear in any anæmia. They may stain deeply or show a considerable central depression and occasionally contain granules or show polychromatophilia.[3] (Plate II, Fig. 15.)

### *B. Cells with Nuclei*

**Normoblasts** are nucleated red cells of the same size as the normal, that is, between 7 and 8 micra in diameter. (Plate II, Figs. 16 to 22.) The nucleus is, as a rule, central and usually single, though often irregular forms are seen, especially the so-called clover-leaf form, or even nuclei with lobulations. These lobular nuclei must be carefully distinguished from nuclei with division figures, with which they have nothing to do. The irregular structure probably indicates a degeneration of the nucleus. Occasionally a cell is seen containing two distinct nuclei, one usually much smaller than the other.

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[1] For further discussion of this subject, see:—*M. Jogichess:* Inaug. Diss. Berlin, 1900.

[2] *Cabot:* Journal of Med. Research, N. S. vol. iv, 1903, p. 15, figures a number of ring-like structures in the red cells and consider them as probably nuclear remnants.

[3] Anisocytosis is a term which has been suggested to designate the condition found in blood in diseases where there is great variation in the size of the cells without reference to their forms.

These normoblast nuclei are distinguished from all others of the same size by their intense staining, and by the fact that the nucleus shows either no nuclear network, only a homogeneous mass of chromatin, or a coarse chromatin arranged more or less radially. Practically the same morphology is shown by the lymphocyte nuclei, especially in pernicious anæmia, when a differential diagnosis is sometimes difficult. The small, deeply staining pycnotic nuclei are sometimes set free in the circulating

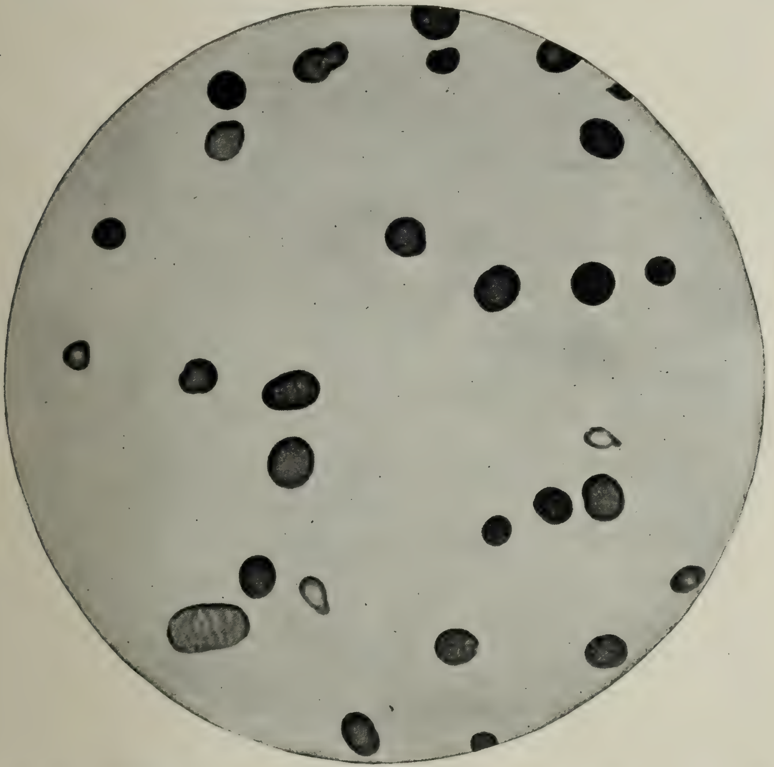

Fig. 30.—Pernicious Anæmia, Showing Poikilocytosis, Anisocytosis, and Wrinkling of the Megalocytes. Magnified 700 diameters.

## DESCRIPTION OF PLATE II

*Stained by the Jenner Method*

**1.** Normal red cell.[1]

**2.** and **3.** Anæmic red cells.

**4.** Crenated red cell.

**5.** Normal sized cell with excess of hæmoglobin. From blood of pernicious anæmia.

**6** and **7.** Polychromatophilic red cells.

**8.** Polychromatophilia with vacuolar degeneration.

**9, 10, 11,** and **12.** Various types of granular degeneration.

**13.** Macrocyte.

**14.** Microcytes.

**15.** Poikilocytes.

**16.** Normoblast with well-marked chromatin network such as is seen in infant blood or anæmia from hæmorrhage.

**17.** Normoblast with pycnotic nucleus. From case of chlorosis.

**18.** Normoblast showing karyorrhexis.

**19** and **20.** Normoblasts showing karyolysis.

**21.** Microblast.

**22.** Poikiloblast.

**23.** Orthochromatic megaloblast with well-marked chromatin network in the nucleus.

**24** and **25.** Polychromatophilic megaloblast corresponding to Engel's metrocyte of the first generation.

**26.** Polychromatic megaloblast. Cell body undergoing solution in the blood plasma.

**27.** Megaloblast with small nucleus corresponding to Engel's metrocyte of the second generation.

**28.** Megaloblasts showing karyorrhexis.

**29.** Megaloblast with pycnotic nucleus.

**30.** Megaloblast with nucleus undergoing karyorrhexis.

**31.** Megaloblast with pycnotic nucleus.

**32.** Very large polychromatophilic megaloblast (Gigantoblast).

**33, 34, 35, 36, 37.** Different stages of mitosis in a normoblast.

**38.** Transitional leucocyte.

**39, 40, 41,** and **42.** Polynuclear neutrophile leucocytes.

**43.** Polynuclear cell of the neutrophile type but without granulations.

**44, 45,** and **46.** Cells with eosinophile granulations as found in normal blood.

**47, 48,** and **49.** Cells with basophile granules as found in normal blood.

**50, 51, 52, 53, 54, 55, 56,** and **57.** Various types of lymphocytes as found in normal blood.

**58.** Large mononuclear cell.

**59** and **60.** Large lymphocytes.

**61.** Indeterminate type of cell resembling a large lymphocyte or a polychromatic megaloblast, often called "irritation form."

**62.** Small lymphocyte containing hæmoglobin.

**63.** Normoblast with a polychromatophilic cell body.

**64** and **65.** Free nuclei from small lymphocytes.

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[1] Plates II to IX are drawn to a scale of 1,000 diameters, so that 1 millimeter equals 1 micron.

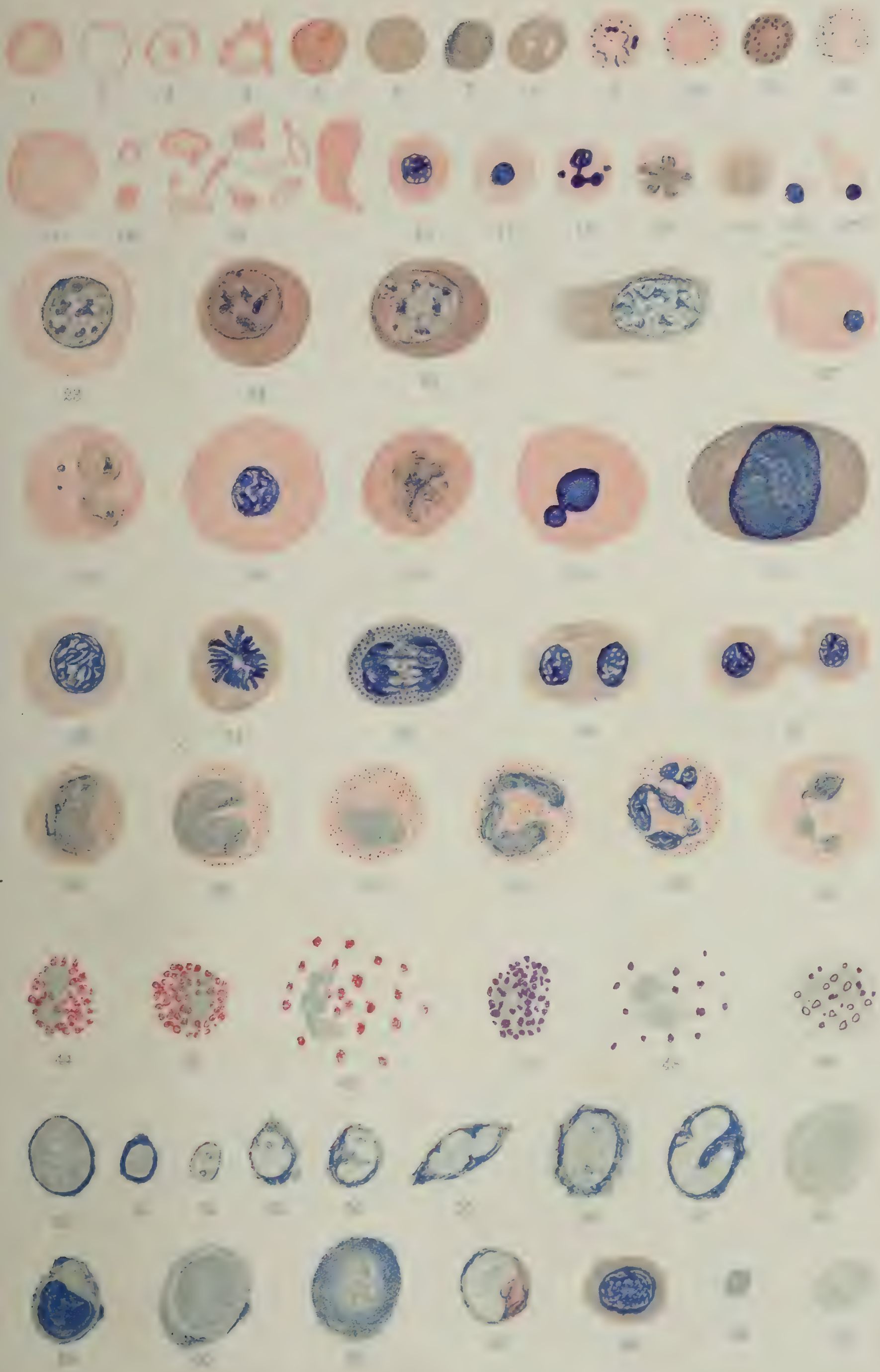

PLATE II.—TYPES OF BLOOD CORPUSCLES.

blood, either by extrusion from the body of the red cell or by the gradual dissolution of the cytoplasm, and may be recognized by the peculiar depth of stain and small size. Mitotic figures are not infrequently found in the nuclei of normoblasts, especially in acute lymphatic and myelogenous leukæmia and in the pseudoleukæmic anæmia of children. Under these conditions the protoplasm of the cell body often shows a combination of granular degeneration and polychromatophilia which causes it to resemble very much the protoplasm of the large lymphocytes. (See Figs. 61 and 63, Plate II.)

**Megaloblasts** are large red cells, two to four times as large as the average red cell. The nucleus is larger than that of the normoblast and contains, as a rule, a well-marked chromatin network, which is especially well brought out in specimens stained either with Jenner or with hæmatoxylin (Figs. 31 and 32). The margin of the nucleus is often very vague and indefinite, shading off quite gradually into the cell body. This difficulty of distinguishing between the nucleus and cell body is increased by the fact that while the latter is often orthochromatic it may be highly poly-

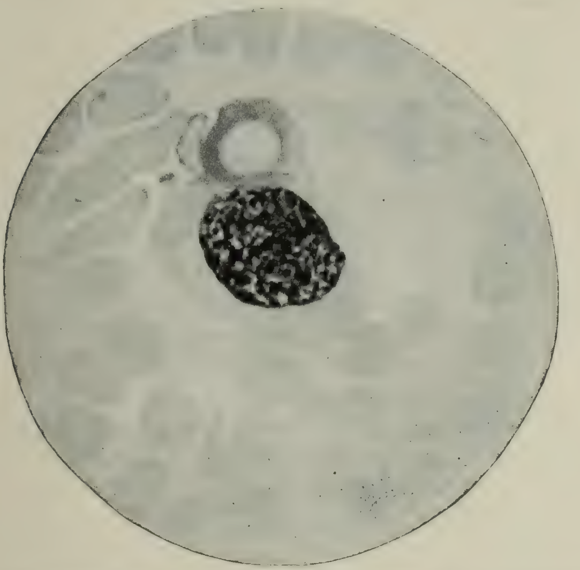

FIG. 31.—MEGALOBLAST SHOWING CHROMATIN NETWORK IN THE NUCLEUS. Magnified 1,000 diameters.

chromatophilic, while the nucleus, as a rule, has much less affinity for basic dyes than the normoblastic nucleus. Punctate degeneration of the body of the cells is occasionally present. The general shape of the cell is circular, although oval and irregular forms occur. Especially in the larger cells—the so-called gigantoblasts—there is often seen a peculiar vacuolation of the cytoplasm.

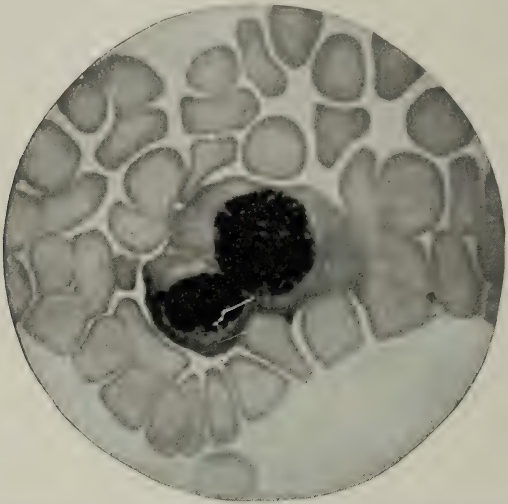

FIG. 32.—TWO MEGALOBLASTS WITH DENSE CHROMATIN NETWORK IN NUCLEI. RESTING STAGE. Magnified 1,000 diameters.

There is occasionally considerable difficulty in distinguishing between a large normoblast and a small megaloblast. The two points of difference are: the normoblast rarely exceeds 10 micra in diameter and the nucleus shows little or no chromatin network, while the megaloblast is over 10 micra and its chromatin network is well marked, especially with the Jenner stain.

Occasionally, however, the nucleus of the megaloblasts becomes compact and may even assume the pycnotic or clover-leaf type so frequently seen in normoblasts (Fig. 33). Pappenheim is inclined to the opinion that all these cells should be classed as normoblasts regardless of the size of the cell body; but from a diagnostic point of view it is better to adhere to the classification based upon the size of the cell rather than upon the structure of the nucleus, which is often difficult to make out. Engel[1] points out the morphological resemblance of the megaloblasts with pycnotic nuclei to the red cells found in the fœtal blood and termed by him metrocytes of the second generation; the metrocytes of the first generation being the large, often polychromatic megaloblasts found in the liver of the fœtus and in advanced pernicious anæmia and occasionally showing mitotic figures. (Plate II, Figs. 23 to 32.)

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[1] Klin. Untersuchung des Blutes, Berlin, 1902.

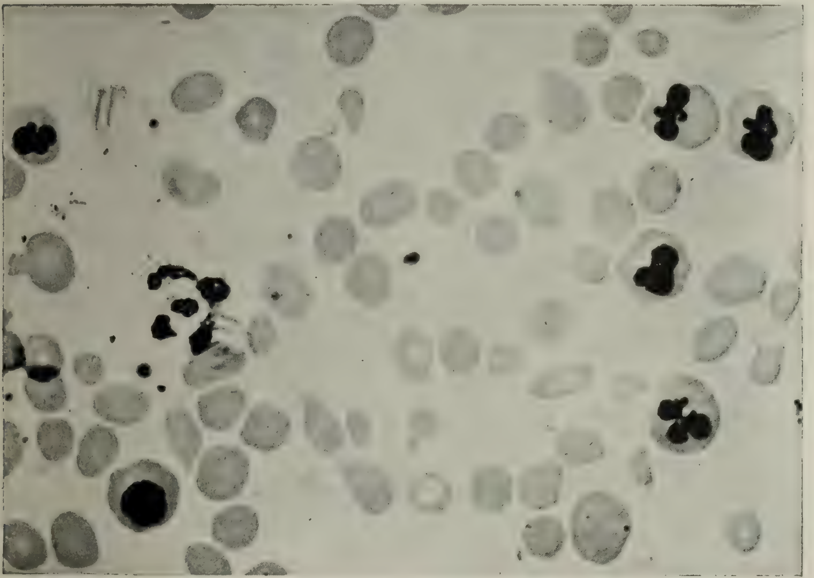

FIG. 33.—MEGALOBLASTIC CRISIS FROM A CASE OF PERNICIOUS ANÆMIA. THE NUCLEI ARE PYCNOTIC AND SHOW NO CHROMATIN NETWORK. Magnified 700 diameters.

Microblasts are very small nucleated cells, which occur but rarely in the blood, but may be occasionally seen in leukæmia and post-hæmorrhagic anæmia. (Plate II, Fig. 21.)

### BASOPHILE SUBSTANCES IN THE RED CELLS

The body of the red cell occasionally stains more strongly with basic dyes than normal. This basophilia appears in three forms:

1. Diffuse, homogeneous basophile staining of the cytoplasm of the red cells, called also polychromatophilia.
2. Basophilic granules scattered through the otherwise normal cytoplasm.

3. So-called stippling or malarial dotting of the red cell.

The first two conditions may be considered together, for where one is present in the blood to a marked degree the other is also, with the exception of lead-poisoning, where the granular degeneration of the red cells is the prominent feature of the blood changes and the diffuse degeneration may be small in amount. Apart from lead-poisoning, the appearance of this degenerative change in the erythrocyte cell body is common in pernicious anæmia, leukæmia, and many of the secondary anæmias, even in chlorosis. It is sometimes well marked in malaria. Granular degeneration can also be induced by poisoning with copper, cobalt, thallium, arsenic, or by inhaling carbon monoxide.[1]

The change is best made evident by such stains as methylene blue, thionin, or hæmatoxylin, and is not well shown in preparations colored with the triacid stain. It appears in either nucleated or non-nucleated cells, usually to a more marked degree in nucleated cells. When no nucleus is present the cell is quite often perfectly normal in size and shape. The individual granules may be quite coarse and few in number or very fine and abundant. They usually occupy the periphery of the cell. As a rule, large and small granules do not exist together in the same cell. Occasionally the whole cell takes a diffuse stain; that is, it is polychromatophilic, while scattered through the degenerated cytoplasm are large numbers of coarse or fine granules. The form of these granules is quite variable: they may be round, angular, or rod-shaped; they frequently resemble small pneumococci. Rarely ring-shaped forms may be observed, which mimic very closely the early ring forms of the malarial parasite. (Figs. 6 to 12, Plate II.)

Their origin is probably twofold: a degeneration of the protoplasm of the red cells, and a breaking up of the nucleus of the red cell (karyorrhexis). (Figs. 28 and 30, Plate II.) In lead anæmia the first condition is the more probable, for often the punctate degeneration is the only change noted in the blood, and may be absent from the red cells of the bone marrow, indicating that the condition is due to a toxic necrosis taking place in the peripheral circulation, and not due to the action of the lead on the young cells of the bone marrow or their precursors, the normoblasts. The opposite is true in artificial phenylhydrazin anæmia in rabbits and in pernicious anæmia and leukæmia in man, for, under these conditions, the bone marrow is filled with polychromatic cells with ragged-edged and degenerating nuclei, while in many of the cells the process of the breaking down of the nucleus and the distribution of its chromatin throughout the cell body can easily be seen in all its stages. In these diseases also, the degeneration is most marked in the nucleated cells of the peripheral circulation, while in lead-poisoning the change appears in cells otherwise normal, and nucleated corpuscles may be absent from the blood. Some observers[1] are inclined to consider both these changes as purely regenerative in nature, and Schmidt[2] has developed experimental evidence of considerable value which points toward the regenerative character of the changes in the red cells of which the granules are the expression.

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[1] *Keil:* Inaug. Diss., Rostock, 1901.

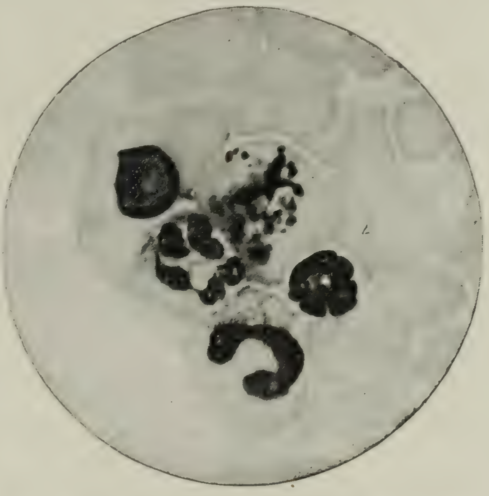

Fig. 34.—Karyorrhexis in a Megaloblast. Pernicious Anæmia. Magnified 1,000 diameters.

*Basophilic stippling* of the red cells, when the latter are stained by a dye containing methylene azure, is frequent in erythrocytes invaded by the parasite of tertian malaria, and may be seen also, after appropriate fixation, in æstivo-autumnal fever,[3] but not in quartan. The appearance has been considered as indicating an early stage of granular degeneration, but it is much more probable that it is due to a coagulation of the proteid of the red corpuscle made evident by the overstain taken up by the nodal points of the coagulum. The writer has seen stippling and granular degeneration in the same red cell. The granules do not stain with the ordinary basic dyes, but only with azure stains, in distinction to the punctate granules which take either stain. (Plate IX, Figs. 28, 44. 47.)

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[1] *Cabot:* Journal of Med. Research, vol. iv, 1903, p. 15.
[2] *Schmidt:* Exp. Beit. z. Path. d. Blutes, Jena, 1902.
[3] *Argutinsky:* Cent. f. Bakt., Bd. xxxiv, 1903, p. 144.

### MORPHOLOGY OF THE BLOOD PLATES

These bodies are small, amœboid, oval, or spherical masses, from 1 to 3 micra in diameter, which are present in large numbers in freshly shed blood. During coagulation they collect to form masses from which the fibrin network radiates. With stains containing methylene azure a distinct central red mass can be demonstrated, which is by some considered as a nucleus (Plate III, Fig. 90). Their number is difficult to determine, because of the rapid formation of clumps, which takes place as soon as the blood is shed. Different observers give from 200,000 to 860,000 per cubic millimeter.

Among the various views as to the origin of the blood plates may be considered the following:

1. That they are derived from the red cells.
2. That they are derived from leucocytes.
3. That they are independent nucleated bodies.

The last view has gained some support from the fact that these minute bodies show faintly staining chromatin masses in their centers when colored with eosin-methylene azure; and that they have independent motion. Their relations to coagulation have been generally considered as mechanical; but recently some observations have been made which point to the blood plates as the source of some of the substances which give rise to clotting. Morawitz[1] has recently reviewed the subject, and in his opinion the blood plates give origin to that portion of the fibrin ferment which has been named thrombogen, the combination of which with the thrombokinase and calcium salts is the body usually known as fibrin ferment. After shedding the blood the thrombogen is activated by the thrombokinase, which may be derived from the tissue juices, and in the presence of lime salts clotting takes place.

No facts of clinical value have been as yet made out from the study of the blood plates.[2]

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[1] Deut. Arch. f. klin. Med., Bd. lxxix, 1904, p. 1.

[2] For a general review of the subject, see:—*Schwalbe:* Lubarsch and Ostertag's Ergebnisse d. Allg. Path., 1904.

## MORPHOLOGY AND RELATIVE PROPORTIONS OF THE LEUCOCYTES

### TYPES OF LEUCOCYTES IN NORMAL BLOOD

**Lymphocytes.**—These are cells with a very narrow cell body which surrounds a relatively large, round, concentric or only slightly eccentric nucleus. They vary in size from about the diameter of a red cell to twice that size. The small lymphocytes are found chiefly in the normal blood of adults, the large ones in the normal blood of children, in lymphatic, and occasionally, in myelogenous leukæmia. The cell body stains more deeply with the basic dyes—such as methylene blue—than the nucleus, and surrounds the latter, in the smaller lymphocytes, as a mere narrow ring. In the large lymphocytes the cell body is wider and often shows a granular, irregularly stained network which simulates true granulation. The periphery of the cell is frequently quite ragged ; sometimes without distinct projections, at others with large masses jutting out from the border. These masses may sever their connection with the cell and appear free in the blood, especially in lymphatic leukæmia. The nucleus is usually spherical, but it may have a sharp indentation on one side or, in lymphatic leukæmia, may break up into irregular nuclei. With the triacid stain the nucleus usually shows but little structure and is faintly stained; the cell body is usually invisible, or seen only as a faint pink ring. With the Jenner stain, on the contrary, the nucleus of the larger forms frequently shows a nuclear network and nucleoli, and the cell body is usually deeply stained and of a dark-blue color, unless the slide has been washed in tap water, when the body of the cell is often paler than the nucleus. (Plate II, Figs. 50 to 65, Plate III, Figs. 80 to 87.) With stains containing methylene azure the nucleus is colored a very deep carmine or reddish purple, showing a coarse chromatin network, while the cell body takes a pale Prussian blue color, deeper in the small forms and lighter in the large lymphocytes and the large mononuclears.

A few of the lymphocytes in health and a considerable proportion in certain severe anæmias show scattered granules. These do not stain with the triacid, but can often be seen in Jenner preparations and more perfectly still after staining with eosin-methylene azure. The exact status of these granules has not as yet been determined. Michaelis and Wolf[1] are inclined to regard them as of the same importance as the other types of granulations, but Ehrlich suggests that they are degenerative in nature. The writer has noted them most often in severe anæmias. They were especially numerous in the lymphocytes of a case of fatal aplastic anæmia following thrombosis of the renal vessels. In leukæmia, where there is much karyorrhexis of the lymphocyte nuclei, these granules are apt to be very abundant in the necrosing cells, and very often fragments of the nucleus can be seen budding out into the protoplasm of the cell body and taking the same stain as the granules which surround it. (Plate III, Figs. 83 to 87.)

The lymphocytes form 22 to 25 per cent. of the white cells of normal adult blood, but are somewhat more abundant in the blood of the new-born. They are derived from the lymphoid tissue in the body, including that of the bone marrow.

**Large Mononuclear Cells.**—These are large cells, two to three times the diameter of the erythrocytes, with a large oval eccentric nucleus and a rather abundant cell body. The nucleus stains more deeply than the cell body, the latter in triacid preparations being visible only as a mere transparent area against the faint diffuse stain of the serum of the blood smear, while the Jenner stain gives a pale blue body, and methylene azure a robin's-egg or Prussian blue. The cell body may contain a few granulations when stained with methylene azure. These cells form only 1 per cent. of the total leucocytes in normal blood, and are produced in the bone marrow. By many observers they are classed as large lymphocytes, while others consider them to be the primary cell of the bone marrow or myeloblasts.[2] They are more abundant in the blood of young children, often rising to 5 or 10 per cent. (Plate II, Fig. 58, Plate III, Figs. 82, 83.)

**Transition Forms.**—These cells resemble the large mononuclear cells, except that the nucleus shows a tendency to take a horseshoe shape and to stain more deeply, while neutrophilic granules are present in small numbers in the cell body. They form 2 to 4 per cent. of the white cells, and are best enumerated with the polynuclear neutrophiles, and not considered as a separate group when making differential counts. (Plate II, Fig. 38.)

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[1] Virchow's Archiv, Bd. clxvii, 1902, p. 151.

[2] *Naegeli:* Deut. med. Woch., 1900, p. 287.

**Polynuclear Neutrophile Cells.**—These cells are sharply distinguished from the preceding types by the irregular form of the nucleus and by the presence of small neutrophile granules in the cell body. (Plate II, Figs. 38 to 43.) They are slightly larger than the average red cell.

The nucleus is usually of an S shape, but may consist of three or four oval masses connected by fine chromatin threads, which may be covered by the granular cell body, so that they appear as discrete nuclei; in some cases, however, the nucleus does break up into three or four small, round, individual nuclei, especially in fresh exudates or in the blood of very severe anæmias and acute infectious diseases. The nucleus stains rather deeply with basic dyes, while the protoplasm of the cell body stains with the acid dyes. The cell body is alkaline in reaction, though less strongly so than that of the lymphocytes. They are formed in the bone marrow from the myelocytes, and are present in the circulating blood to about 65 to 75 per cent.

**Eosinophile Cells.**—These cells are little larger than the erythrocytes, and are characterized by their very coarse, highly refractile granules, which stain strongly with acid dyes, such as acid fuchsin or eosin. The nucleus takes the basic dyes rather faintly, but otherwise its morphology is that of the polynuclear neutrophile form. The periphery of the individual eosinophile granules is often more deeply stained than the center. These cells are the most actively amœboid of the leucocytes. They form, on the average, from 2 to 4 per cent. of the leucocytes in the blood, with .6 to 11 per cent. as the upper and lower limits in healthy persons.

**Basophile Cells (Mast Cells).**—These exist in normal blood in rather small numbers, not above .5 per cent. They are about the size of the red cell, with a faintly staining cell body and irregularly shaped granulations, which stain deeply with the basic dyes. The color which the granules take, for instance, with the Jenner stain, is often a selective one; that is, while the nucleus takes the pure blue of the methylene blue, the granulations are

## DESCRIPTION OF PLATE III

*Stained by the Jenner Method*

*Figure A*

**66, 67, 68, 69, 70, 71.** Various types of myelocytes from a case of myelogenous leukæmia.

**72.** Unripe eosinophile myelocyte containing a few basophilic granules.

**73, 74,** and **75.** Eosinophile myelocytes from a case of myelogenous leukæmia.

**76.** Basophile cell with small granulations.

**77.** Basophile cell containing two types of granules, one eosinophilic, the other basophilic.

**78.** Basophile myelocyte with coarse granules.

**79.** Basophile myelocyte with scattered granules.

**80, 81,** and **82.** Cells belonging to the large mononuclear group, by some considered lymphocytes.

**83, 84, 85, 86,** and **87.** Lymphocytes containing reddish granules; considered by some as of the same importance as eosinophile, neutrophile, and basophile granules, by others as products of degeneration of the nuclear chromatin.

**88** and **89.** Degenerated cells ("basket" cells).

**90.** Blood plates showing central mass of nuclear substance.

### *Figure B.*—NORMAL BONE MARROW

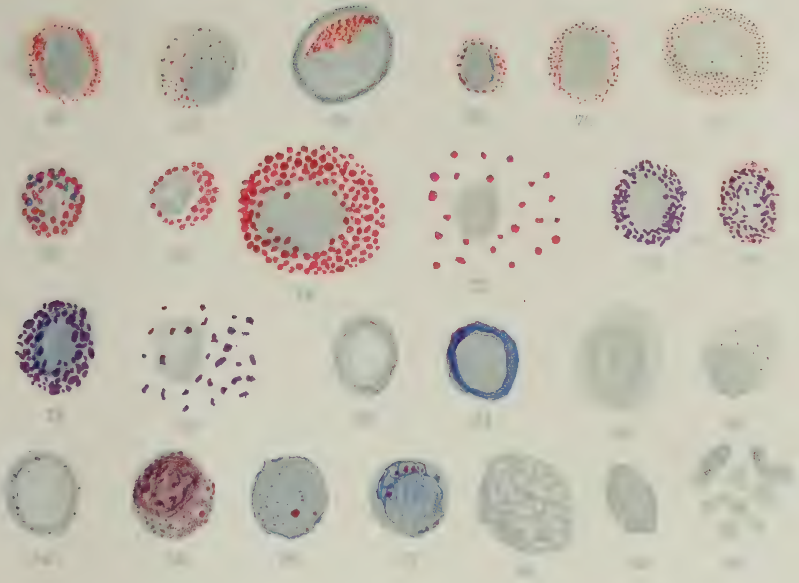
The red cells are the same as those found in normal blood, with the exception of the fact that numerous normoblasts are present. Some of these normoblasts have large nuclei with well-marked chromatin network and either orthochromatic or polychromatic protoplasm. In some of the cells, however, the nucleus is small, pycnotic, and round, or it may assume a dumb-bell or clover-leaf form.

Polynuclear neutrophiles, eosinophiles, and basophiles are present. They do not differ from cells of the same type found in the circulating blood.

Mononuclear eosinophiles, neutrophiles, and basophiles are also present in large numbers. They are the same type of cell as the myelocytes found in the blood in myelogenous leukæmia.

Besides the well-formed myelocytes there are often immature types, either with no granules or with a few imperfectly formed granules. These cells have been termed myeloblasts or primary marrow cells. Three of these are figured in the left-hand portion of the plate; one contains a double nucleus and is surrounded by a very deeply staining cell body. In the other two, the cell body stains much more faintly than the nucleus. A fourth form is figured at the lower right-hand corner of the plate. The nucleus is large and oval and frequently contains nucleoli. The cell body is basophilic and shows a coarse network, though no granules are present.

A moderate number of lymphocytes are usually present in the marrow, and multinuclear cells, one of which is figured in the lower left-hand corner, are occasionally found in smears of the bone marrow and rarely appear in the blood as an agonal phenomenon. Numerous poorly staining free nuclei are present in all specimens of marrow. Several of these are figured in the lower left-hand corner just above the multinuclear cell.



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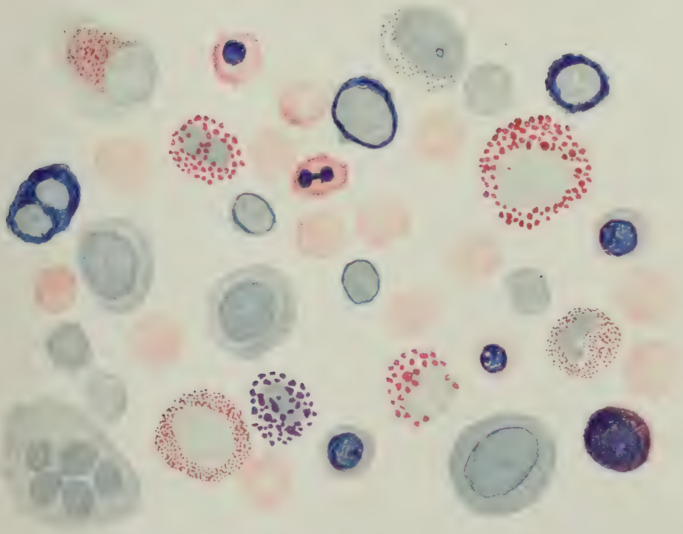

B

PLATE III.—A. TYPES OF BLOOD CORPUSCLES.
B.—NORMAL BONE MARROW.

violet or purple. These granulations are rather variable in their number, shape, or size. Many cells contain only a few; they are often oval, and in faintly stained specimens the center of the granule is fainter than the periphery. The nucleus is often covered by the granules, so that its shape is difficult to make out. In triacid preparations these granulations are not, as a rule, visible, occasionally, however, a few granules take a violet color.

## TYPES OF LEUCOCYTES FOUND IN THE BLOOD IN DISEASE

**Myelocytes with Neutrophile Granules.**—These are large cells, measuring at least 15 micra in diameter, with a large, oval, feebly staining nucleus which is generally slightly eccentric. They are differentiated from the large mononuclear cells of normal blood by the presence of neutrophile granules in the cell body. (Plate III, Figs. 66 to 71.) In some of the early forms which appear in very severe leukæmias or in the bone marrow, there are no neutrophile granules, but the cell may be distinguished by its strongly basic staining power, while the cell body of the large mononuclear, as it appears in normal blood, is more faintly stained. This distinction does not appear, however, in specimens stained by Ehrlich triacid stain. They possess but slight powers of amœboid movement.

These cells appear in the blood abundantly only in splenomyelogenous leukæmia. In small numbers, however, they may be found in the blood of patients suffering from tumors involving the bone marrow, either directly or by metastatic deposits; more abundantly in the latter case. They are also seen in pernicious anæmia, in acute lymphatic leukæmia, and in severe secondary anæmias, in severe forms of diphtheria, during septic infections with a large leucocytosis and especially in variola, where they frequently occur in the peripheral blood, and after the crisis in pneumonia. In diphtheria their presence in large numbers is of distinctly bad prognosis.

**Myelocytes with Eosinophile Granules.**—These are large mononuclear cells similar to the preceding, but with coarse, eosinophile granulations. They are frequently found of small size in leukæmia, and also furnish excellent examples of the degenerative forms often met with in smears from advanced cases of leukæmia, where a pale, feebly staining, large nucleus is surrounded for a distance of 20 to 30 micra by scattered eosinophile granules. They occur practically only in leukæmia, in connection with tumors involving the bone marrow; and in the pseudoleukæmic anæmia of infants. (Plate III, Figs. 72 to 75.)

**Myelocytes with Basophile Granules.**—These are large mononuclear cells with basophilic granules, and are seen only in long-standing severe myelogenous leukæmias. They also appear as degenerated forms with widely scattered granules, similar to the eosinophilic myelocytes. (Plate III, Figs. 76 to 79.)

**Small Mononuclear Neutrophile Cells.**—These are the cells which are smaller than the small myelocytes. They are formed by fragmentation of the polynuclear neutrophiles in certain acute diseases, and are rarely seen. Ehrlich describes them in a case of hæmorrhagic small pox, and they may be found also in fresh exudates.

**Large Mononuclear Cells (Irritation Forms)** which appear in the blood under the same conditions as the myelocytes. The cell is larger than the normal red cell, often as large as the large mononuclears of normal blood. The nucleus stains with moderate depth. No network is apparent with the Ehrlich triacid stain; the cell body stains a dull brown and contains no granules. With the Jenner stain the nucleus shows a moderately well-marked network, the cell body stains more deeply than the nucleus and of a pale blue. These cells sometimes resemble closely the large nucleated red cells seen in severe chronic leukæmia, pernicious anæmia, and the bone marrow of infants, but the latter type of cell stains more intensely blue, both in nucleus and cytoplasm, and the cell body is much more regular in its outline and of a more homogeneous texture. Türk [1] is inclined to regard them as belonging to the lymphocyte group. (Plate II Fig. 61.)

**Rare and Irregular Types of Cells.**—In advanced cases of leukæmia and pernicious anæmia, cells appear in the blood which are often difficult to identify; they are often without granules and do not fit into any of the above classes. Myelocytes are often seen containing no granules, or only a few neutrophile ones. (See under myeloblasts in the paragraph on bone marrow.) While their identification must await the growth of our knowledge on the subject of the blood, yet from a clinical and diagnostic standpoint they are of little importance, as they appear only in cases

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[1] Verhalten des Blutes bei Acuten Infectionskrankheiten. Wien, 1898, p. 115.

The following table, modified from DaCosta, shows the chief points concerning the staining reactions of the leucocytes:

TABLE OF DIMENSIONS AND STAINING REACTIONS OF THE LEUCOCYTES

| Type of Cell. | Size. | Color. | Ehrlich Stain. | Jenner Stain. | Methylene-Azure Stain. |
|---|---|---|---|---|---|
| Small lymphocyte......... | 5–7 $\mu$. | Nucleus. | Dark green or blue. | Pale blue showing nucleoli. | Dark blue or purple. |
| | | Cell body. | Pale pink. | Deep blue. | Pale Prussian blue or greenish blue. |
| Large lymphocyte. ....... | 7–15 $\mu$. | Nucleus. | Dark green or blue. | Pale blue. | Dark blue or purple. |
| | | Cell body. | Pale pink. | Deep blue. | Pale Prussian blue. |
| Large mononuclear leucocyte. | 10–15 $\mu$. | Nucleus. | Pale green or blue. | Pale blue. | Dark blue or purple. |
| | | Cell body. | Unstained or pale pink. | Pale blue. | Pale Prussian blue. |
| Transitional leucocyte..... | 10–15 $\mu$. | Nucleus. | Pale blue. | Dark blue. | Dark blue or purple. |
| | | Cell body. | Pink with a few neutrophile granules. | Bluish or pink with a few neutrophile granules. | Pink with a few neutrophile granules. |
| Polynuclear neutrophile... | 6–15 $\mu$. | Nucleus. | Dark blue or green. | Dark blue. | Dark blue or purple. |
| | | Cell body. | Pink with numerous neutrophile granules. | Pink with numerous neutrophile granules. | Pink with numerous neutrophile granules. |
| Eosinophile.... | 6–15 $\mu$. | Nucleus. | Pale blue or greenish. | Pale blue. | Blue or purple. |
| | | Cell body. | Pink with numerous coarse reddish granules. | Pink with numerous coarse reddish granules. | Pink with numerous coarse reddish granules. |
| Polynuclear basophiles.... | 7–12 $\mu$. | Nucleus. | Very pale blue or green. | Pale blue. | Pale blue or purple. |
| | | Cell body. | Unstained; occasionally shows a few bluish granules. | Unstained or pale pink with brilliant purple granules. | Unstained or pale pink with reddish granules. |
| Myelocyte..... | 10–25 $\mu$. | Nucleus. | Pale blue or green. | Blue. | Dark blue or purple. |
| | | Cell body. | Unstained, containing numerous eosinophile or neutrophile granules. | Pale pink or blue, containing numerous neutrophile, eosinophile, and basophile granules. | Pale pink or blue, containing neutrophile, eosinophile, and basophile granules. |
| Irritation forms (Türk).. | 7–15. | Nucleus. | Deep blue or green. | Pale blue. | Blue or purple. |
| | | Cell body. | Dark brown. | Dark blue. | Dark blue. |

when the blood changes are so advanced that a diagnosis can be easily made. Cells of large size, with one or many nuclei, are occasionally found in the peripheral circulation in cases of leukæmia, in eclampsia, and in severe acute infections with marked leucocytosis. They are the giant cells normally found in the bone marrow. (Plate III, Fig. B.)

**Degenerated Cells.**—Basket cells or "shadows." We may occasionally see in normal blood degenerated leucocytes which stain poorly and show no granules. In a more advanced stage of the same process the cell is represented merely by a meshwork composed largely of the remnants of the nuclear chromatin. Such degenerated and swollen cells are frequently met with in severe anæmias and especially in all types of leukæmia where large numbers of white cells are being destroyed, as is shown by the high excretion of the end products of nuclear substances in the form of the purin bodies. (Plate III, Figs. 88 and 89.)

## THE BLOOD IN INFANCY AND CHILDHOOD

The blood of healthy children shows certain variations from that of adults which are worthy of notice and which should be taken into consideration in the diagnosis of blood changes in infants.

**Number of Red Cells.**—The average number of red cells in the blood of healthy infants is, as a rule, considerably higher than in adults. During the nursing period it averages about 5,580,000, with a maximum during the first week of life. During the second year the number is about the same as during the first year, 5,680,000. From the second to the sixth year the average count is rarely under 5,900,000, girls showing a slightly lower count. Physiological variations in successive counts are frequently met with in nursing infants and in young children, fluctuations of a million cells being occasionally found.

**Hæmoglobin.**—The hæmoglobin is high for a few days after birth, and then sinks, so that it is lower in the first year of life than later, and also shows considerable variations, the absolute amount present varying from 11.5 grams to 13.5 in 100 grams of blood,[1] or according to Perlin, from 58 to 78 per cent. by the

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[1] *Karnizki:* Archiv für Kinderheilkunde, 1903, p. 42.

Fleischl-Miescher hæmoglobinometer. After the nursing period there is usually a gradual increase, the hæmoglobin rising to an average of 13 grams, or 75 to 85 per cent. in the sixth year, until the tenth year, when the amount reaches the average for adults of 14 to 14.5 grams. The variation between the sexes in the amount of hæmoglobin is very slight. There is usually a relative increase in childhood in the amount as compared with the red cells.

**Specific Gravity.**—In infants during the first few days after birth the specific gravity undergoes rapid fluctuations between 1.060 and 1.080, probably due to venous stasis at the time of delivery and the chilling of the body afterward. The density of the blood does not correspond either with the hæmoglobin or with the number of corpuscles. After this time the variations are less irregular and in the first few months the specific gravity fluctuates between 1.055 and 1.059, the average being 1.056. The limits are 1.053 and 1.060. In males the specific gravity is usually .001 higher than in females. From the second to the fourth years the specific gravity rises to an average of 1.058. In the sixth year it reaches an average of 1.061 where it remains throughout childhood.

**Number of White Cells.**—During the first few days after birth the leucocytes may reach 15,000 to 19,000, while in the nursing period the white cells average 12,000; the maximum being 16,000, and the minimum 8,600. According to Japha,[1] a digestion leucocytosis in a small child may reach 20,000 to 25,000, with 55 per cent. of lymphocytes. During childhood the number of cells diminishes, the average during the period from the first to the sixth year being about 9,000, the maximum, 13,000, the minimum, 6,900. During the period from the sixth to the fifteenth year, the average is 7,900, with a maximum of 12,400 and a minimum of 5,400.

**Morphology of the Red Cells.**—In the blood of healthy nursing infants up to the age of about eight months, it is possible after prolonged searching to find occasional nucleated red cells of two types: one in which the cell is about the same size as that of the normal red corpuscle, usually with a pycnotic nucleus and an orthochromatic cell body; the other from two to four times as large as the normal red cell and with a polychromatic cell body and a nucleus showing a distinct chromatin network.

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[1] Jahr. f. Kinderheilkunde, Bd. lii, 1900, p. 242. See also ibid., Bd. liii, 1901, p. 179.

**Morphology and Relative Proportions of the White Cells.**—During the nursing period, the relative proportion of the lymphocytes varies from 51 to 59 per cent., while the proportion of the neutrophiles is approximately 28 per cent. From the eighth to the tenth month an increase in the absolute and relative number of the lymphocytes is noticeable, the proportion rising to 61 per cent. After the tenth month a fall is again noted to 56 per cent., the neutrophiles slightly increasing during this period to 34 per cent. During the second year the lymphocytes average 55 per cent., the neutrophiles 41 per cent. During childhood there is noted a gradual increase in the number of the neutrophiles toward the proportion which is observed in adults. From the sixth to the eighth year the lymphocytes average 41 per cent., and the neutrophiles 46 per cent. From the tenth to the twelfth year the lymphocytes average 29 per cent., the neutrophiles 56 per cent. From the twelfth to the fourteenth year the lymphocytes average 30 per cent., the neutrophiles 55 per cent. The increase in the neutrophile cells occurs chiefly about the fourth year of life and this increase is still noticeable up to fifteen years of age. Transitional neutrophiles are present during the first few months in a proportion of about 11 per cent., but fall gradually to 7 per cent. at the end of the first year. The eosinophile cells vary during the nursing period between 7.5 per cent. and .5 per cent. During childhood the variations are more considerable, and may reach 12.5 per cent. as a maximum or fall to .7 per cent. Basophiles may vary between .1 and 2 per cent., in other words, show greater fluctuations than in normal adult blood. The large lymphocytes in infants are usually more abundant than in adults. There are no marked differences in the morphology of the granular cells from those of adults.

## BLOOD FORMATION AND BLOOD-FORMING ORGANS

In the study of the various diseases which affect the blood, it is necessary constantly to refer to the organs in which this tissue is produced, inasmuch as the changes in the organs are often primary and those in the blood secondary. As the development of the blood in the embryo varies considerably from that in the adult, and since in certain diseases, notably in pernicious anæmia, there is a return to the embryonic type of blood formation, it is of interest to consider briefly some points in the blood and blood-forming organs during embryonic life.

## BLOOD FORMATION

The earliest formation of red corpuscles of the blood has been noted before the appearance of the rudiments of the liver and spleen, in the very small embryos of some of the mammalia. The cells are apparently produced by the proliferation of the endothelial cells of the small capillaries; or, according to some observers, the early red cells are produced in the substance of large connective tissue cells which later become hollowed out and form a portion of the developing capillaries. This intracellular or intravascular development of red cells ceases with the formation of the liver and lymphatic system.

In the mammalia the development of the blood-forming organs can be divided into two stages, the first of which may be termed the premedullary stage, where the corpuscles are formed in various organs of the body, especially the liver, and a medullary or definitive stage, which begins with the development of the bone marrow at about the third month of embryonic life and continues under normal conditions until death. During this period the bone marrow is, in conditions of health, the only source for the red cells and the granular leucocytes, while the lymph nodes possibly contribute some or all of the lymphocytes.

In the premedullary period the leucocytes play a very unimportant rôle and the circulating blood corpuscles of small embryos of from one-half to one cm. in length are exclusively of the hæmoglobin carrying type. These cells can be divided, as has been shown chiefly by Engel, into two distinct groups, which he has called metrocytes of the first and second generation. The metrocytes of the first generation have large nuclei with well-marked chromatin network, and frequently show mitoses. Those of the second generation have one or two, more or less pycnotic nuclei. The few red cells of this period which contain no nuclei are of large size, corresponding to the macrocytes, which will be further discussed in the subject of pernicious anæmia. The protoplasm of all these cells is usually polychromatic, but occasionally orthochromatic, especially in the smaller corpuscles, either with or without nuclei.

During the second or third month of embryonic development a rapid change takes place in the number of the nucleated cells in the blood, non-nucleated forms rapidly assuming the preponderance and the general type of the cell changing to that which resembles more perfectly the erythrocyte of the normal adult blood; that is, at the end of the third month a smear from the blood of the embryo shows numerous orthochromatic normocytes with moderate numbers of normoblasts, the latter often showing a considerable degree of polychromatophilia. Leucocytes make their appearance in the blood about the tenth week of embryonic life, in the form of cells corresponding to the lymphocytes of adult blood. Rarely one may also find a few myelocytes, chiefly of the neutrophile variety. Embryos of the tenth or twelfth week show occasional polynuclear and mononuclear neutrophiles and eosinophiles in the circulating blood. The lymphocytes remain most numerous during the entire period, and this relative lymphocytosis continues for a number of years during childhood. Immediately before birth the circulating blood contains chiefly orthochromatic red cells which are very rich in hæmoglobin, occasionally showing a moderate polychromatophilia, while a polychromatophilic normoblast is occasionally seen. The lymphocytes are three to four times as numerous as the polynuclear neutrophiles; eosinophile cells are infrequent, and a few myelocytes are present. Shortly after birth the normoblasts practically disappear from the blood, never to reappear except under pathological conditions. The excess of lymphocytes diminishes rapidly; the eosinophiles increase; the myelocytes disappear permanently from the blood and are only to be seen in disease.

## BLOOD-FORMING ORGANS

**The Liver.**—The formation of the red and white cells in specialized organs of the body begins with the development of the liver. Smears from the liver of a ten weeks' fœtus show large numbers of orthochromatic and polychromatic metrocytes, either with large nuclei, containing well-marked chromatin network, or with small pycnotic nuclei; two or three being occasionally present in the cell. It is sometimes difficult to distinguish the polychromatic metrocytes, which correspond to the megaloblasts seen in pernicious anæmia, from large lymphocytes, a difficulty which one also meets with in the anæmias, as the granular bluish cell body of the lymphocyte resembles very closely the cell body of the megaloblasts showing advanced polychromatophilia. At this same period leucocytes may be found in the vessels of the liver, which correspond to the lymphocytes of adult life.

Smears from the liver of a fœtus of five or six months, or in other words, at a period considerably after the development of the bone marrow, still show a marked formation of the red cells in the vessels of that organ. Megaloblasts with large and small nuclei are still present, as well as orthochromatic and polychromatic erythrocytes and normoblasts. Myelocytes are also abundant, and occasionally basophilic cells may be found. Lymphocytes, both large and small, are also present.

**The Bone Marrow.**—Smears from the bone marrow of a fœtus of the second month show no characteristic marrow cells but only the circulating blood of the same composition as that found in the heart or obtained from the umbilical vein.

At the third or fourth month the formation of marrow begins, and smears show numerous red cells, both megaloblastic and normoblastic in type, and myelocytes. Smears made at term demonstrate very beautifully all of the stages of blood development. Especially characteristic of this infantile marrow is the presence of a large number of nucleated red cells with extreme polychromatophilia of the protoplasm, so that they can scarcely be distinguished from the lymphocytes, except by the fact that the nuclear chromatin is arranged in a more delicate network than is usual in leucocytes.

**The Spleen.**—This organ does not take a very large part in the process of hæmatogenesis during embryonic life. Smears from the spleen during the third month show about the same morphology as is seen in the circulating blood, except that lymphocytes are usually more abundant. Myelocytes are fairly numerous. After birth the spleen takes no important part in hæmatogenesis. Smears from the organ show, except for the cells of the blood in circulation in that viscus, only large numbers of lymphocytes, necrotic leucocytes, and red cells. Evidences of phagocytosis may occasionally be made out. It is possible that a certain number of lymphocytes are derived from the splenic follicles.

In pernicious anæmia, however, changes are noted in the organs which suggest, to a certain extent, structures seen in the liver and spleen of young embryos. Deposits of myelocytes are not infrequent in both liver and spleen,[1] and occasionally one finds a large number of dividing red cells, an evidence that the bone marrow is unable to supply the demand for new-formed blood corpuscles, and that a partial return to embryonic conditions has occurred. In certain infectious diseases, especially variola, and in myelogenous leukæmia, the spleen takes on what has been termed a myeloid transformation with the production of large numbers of myelocytes. Some observers regard these as true metastases of a type comparable to those seen in the distribution of growths of a malignant nature. Others are inclined to consider the change merely as a return to the embryonic condition; and it is well known that when a leukæmia is complicated by an intercurrent infection the spleen diminishes in size and myeloid deposits largely disappear. Such a change does not seem likely to occur if these deposits were of true tumor nature.

**Lymph Nodes.**—The lymphocytes of the blood are formed in the lymph nodes and in the lymphoid tissue of the body; and smears from these organs show numerous large and small lymphocytes such as are seen in the circulating blood. A moderate number of degenerated forms are also seen, together with a few red cells. In lymphatic leukæmia the nodes may show a greatly increased number of lymphocytes, and sections of the nodes show the efferent ducts filled with cells of lymphoid structure. Sections of the tonsils and other lymphoid organs also show the lymphocytes penetrating the walls of the blood-vessels and thus entering the circulation.[2] In myelogenous leukæmia the lymph nodes occasionally contain deposits of myelocytes, and in some of the acute infectious diseases the nodes show a moderate number of polynuclear cells, especially of the eosinophile variety. In pernicious and other severe types of anæmia, the sinuses of the nodes are occasionally filled with red cells, which some observers consider as newly formed in this situation. Others assume that the process going on is that of destruction of the red cells. In lymphatic leukæmia it is probable that the chief source of the lymphocytes in the circulation is not the lymph nodes, but the lymphoid tissue of the bone marrow, which under these conditions is transformed into the so-called lymphoid marrow composed largely of lymphocytes with relatively few red cells and granular leucocytes.

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[1] See *Morawitz:* Deut. Arch. f. klin. Med., Bd. lxxvii, 1903, p. 553. Also *Kurpjuweit:* Ibid., Bd. lxxx, 1904, p. 168.

[2] *Mosse:* Zeit. f. klin. Medicin, Bd. l, 1903, p. 70.

**Adult Bone Marrow.**—At birth the diaphyses of the long bones are largely filled with red marrow, but during childhood this is gradually replaced by fat tissue, until finally, during adult life, the cavities of the long bones are wholly filled with fat and the marrow is confined almost entirely to small deposits in the diaphyses and to the cartilaginous ends of the ribs and the bodies of the vertebræ. After severe hæmorrhages and in advanced anæmias, especially of the pernicious type, and in leukæmia, the fatty marrow of the diaphyses may again be filled with a pasty mass of reddish-gray substance which is largely composed of red and white cells and their antecedents in the form of nucleated reds and myelocytes. In some cases, however, the marrow is unable to respond to the demand for new cells, and the patients may die with an extreme anæmia and no hypertrophy of the marrow.

In smears made from the normal adult human bone marrow, preferably stained by the Jenner method, the following cells can be found:

### *Red Cells*

1. The normal red cell as it is found in the circulating blood.
2. Normoblasts with small, deeply staining, so-called pycnotic nuclei, or cells with vesicular or irregular nuclei; sometimes of clover-leaf or rosette shape, sometimes separated into two nuclei usually connected by a slender thread of nuclear substance. These two nuclei are often of unequal size.
3. Very rarely a megaloblast with its large pale nucleus with well-marked chromatin network.

### *White Cells*

A. Forms which appear in the circulating blood in health.

1. Polynuclear neutrophiles and transition forms of normal size, shape, and staining reaction.
2. Eosinophile cells of normal size and shape.
3. Normal-sized basophile cells with irregular nuclei.
4. Lymphocytes, both large and small, with deeply staining

nuclei, well-marked nucleoli and rim of darkly colored cell body. The number of lymphocytes is usually small.

5. Large mononuclear cells with deeply staining nucleus and pale cell body.

B. Forms which appear in the circulating blood only in disease.

1. Myeloblasts. Cells with oval nuclei and a non-granular cell body which is slightly basophilic. The nuclei contain nucleoli; they do not stain as deeply as those of the lymphocytes and are more strongly basophilic than the cell body. The nuclear network is usually well marked, especially with Jenner. They vary in size from that of the small lymphocytes to the diameter of the myelocytes. The smaller forms have but little cell body; the nuclei are concentric. The large types have large oval nuclei, often eccentric, and a relatively large cell body. The nucleus may show a slight lateral incurving, such as is seen in the transition forms of the circulating blood. These cells are, as a rule, confined to the marrow or to embryonic blood, but in severe pernicious anæmias, in leukæmias, and occasionally in typhoid, they may appear in the peripheral circulation. They are probably early forms of myelocytes, though some observers hold that they correspond to the large lymphocyte group and appear in the circulation as the large mononuclears.

2. Neutrophile myelocytes, with one, or, rarely, two nuclei and a varying quantity of neutrophile granulations. When two nuclei are present they are the result of mitotic division without separation of the cell body, and the two large nuclei are left flattened against each other and lying close to the border of the cell. Nucleoli are usually absent with the triacid but may be present with Jenner. The outline of the cell body is often very hazy, and the granules may be spread out over the slide near the cell, and may even lie on some neighboring cell with a different granulation type, and suggest a mixture of two sorts of granulation in the same cell, a condition found in the circulation only in very chronic leukæmias with profoundly altered bone marrow.

3. Eosinophile myelocytes with one or two nuclei. The eosinophile granules are especially liable to be brushed out of the cell by the process of smearing and to be distributed over the slide near the cell. This is not entirely due to the rough treatment the cell undergoes while being smeared, for in sections of the bone marrow which have not been subjected to mechanical in-

fluences other than the action of the fixing fluid, scattered eosinophile granulations may be lying near cell nuclei. In some of the cells the granules may either all take a blue stain with the Jenner or a few granules may assume this color. These granules do not show the metachromatic quality of the mast-cell granules but take a pure blue, which is probably an evidence of youth, the more mature and perfect cells not showing any blue granules. These cells appear occasionally in advanced myelogenous leukæmia. (Plate III, Fig. A, No. 72.)

4. Basophilic myelocytes are rare. They are of the same type as those seen in leukæmic blood.

5. Giant cells (myeloplaxes). These are very large multinuclear cells without granulations and a cell body which takes a deep, diffuse, basic stain. They are constantly present in small numbers in the red marrow, but appear in the blood with extreme rarity in leukæmia, generally just before death. (Plate III, Fig. B.)

6. Besides the type cells described above, the bone marrow frequently contains immature forms of red and white cells which are extremely difficult to identify and class. These are cells with a nucleus resembling that of a lymphocyte, but with a cell body of variable size and staining reactions; cells apparently myelocytes without granules are also seen; and, thirdly, cells whose nuclei are strongly suggestive of the megaloblast nucleus, but whose cell body is very ragged in outline and deeply stained with methylene blue. (Plates III, Figs. A and B.)

### HÆMOCONIEN

Small round colorless granules generally less than one micron in diameter may be seen in the blood of persons both in health and in disease. These bodies, which were described by Müller,[1] are highly refractile and have rapid Brownian motion in the serum. They are insoluble in alcohol, ether, and acetic acid, and are not blackened by osmic acid. They have no connection with the formation of fibrin. Müller describes these bodies as a new organized constituent of the blood, but they are generally considered as possibly free neutrophile or eosinophile granules.[2] They are as yet of no clinical importance.

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[1] Cent. f. Allg. Path., Bd. vii, 1896, p. 529.

[2] *Stokes* and *Wegefarth :* Johns Hopkins Hospital Bulletin, 1897.

## IV. LEUCOCYTOSIS

Leucocytosis is usually defined as a transitory increase in the number of white cells in the circulating blood, but it is convenient for practical reasons to divide leucocytoses into *relative* and *absolute.*

A relative leucocytosis occurs when the proportions between the various types of leucocytes are in any way altered without any marked changes in the total number of leucocytes in the peripheral blood; in other words, when there is an increase in the number per cubic millimeter of any type of cell.

An absolute leucocytosis, on the other hand, occurs when there is an increase in the number of leucocytes in the peripheral blood, over the normal limit of ten thousand in adults, without regard to any change which may take place in the proportions of the various types of leucocytes present. Thus, in typhoid fever the number of leucocytes is diminished, while the relative proportion and absolute number of the small lymphocytes is increased—in other words, there is a relative lymphocytosis.

We may also classify leucocytoses as *hypoleucocytoses* (*leucopenia*) and *hyperleucocytoses.* In the first, the number of leucocytes is diminished in the peripheral blood, either by reason of a diminished productive capacity of the bone marrow and lymph tissue, or because of some negative chemotactic substance in the blood which causes the leucocytes to collect in the capillaries of the internal organs. The best example of such action is seen in typhoid fever,[1] in measles, or in lobar pneumonia of an extremely severe type, where very few leucocytes may be found in the blood. By injecting into animals the toxins of some of the pathogenic bacilli, a primary reduction may be observed to take place in the leucocytes in the peripheral blood, to be followed in a few hours by a rapid rise in number to a point far above the normal. The leucocytes in such cases are found to be collected chiefly in the capillaries of the lungs.

As a rule, such a primary hypoleucocytosis is not observed in human infections, because blood examinations are not made at so early a stage of the disease as symptoms of the infection may not appear until later.

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[1] *Blum:* Leukopenische Blutbefunde bei Infektionskrankheiten. Wien. klin. Woch., 1899, Bd. xii, p. 401. *Kast* u. *Gütig:* Deut. Arch. f. klin. Med., Bd. lxxx, 1904, p. 105.

The true hyperleucocytoses are incited by a positive chemotactic action which causes a particular type of leucocyte or all types of leucocytes to appear in larger numbers in the blood than is normal. The best example of such a condition is seen in pneumonia of the lobar type due to the pneumococcus, and in suppuration due to the strepto- and staphylococci when the products of the bacterial metabolism are being rapidly absorbed. The stimulus of these toxins probably acts on the bone marrow and causes the leucocytes already formed to appear in the circulation and also incites further leucocyte production. At any rate, during the initial rise of a marked leucocytosis, especially in children, a large number of immature leucocyte forms can be found in the blood, including myelocytes and nucleated red cells, a condition which indicates that the marrow has been unable to supply a sufficient number of mature cells to the circulation. After prolonged infections, a hyperplasia of the bone marrow is incited with replacement of large areas of the fatty tissue in the bone cavities by medullary cells.[1]

The leucocytoses may be classified still further into *polynuclear neutrophile* and *eosinophile* leucocytoses, *basophile* leucocytoses, *lymphocyte* leucocytoses or *lymphocytoses*; while some observers speak of a mononucleosis due to an increase in the large mononuclear cells, but owing to the difficulty of distinguishing these cells from large lymphocytes, this separate type of leucocytosis is not generally accepted.

## PHYSIOLOGICAL LEUCOCYTOSES

**Physiological polynuclear neutrophile leucocytosis** may occur under purely physiological conditions, such as the ingestion of food, after exercise, after cold baths, and during pregnancy.[2] The increase in the number of the leucocytes is, however, not very great, usually not rising higher than twelve to fourteen thousand.

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[1] See for further details:—*Rubinstein:* Zeit. f. klin. Med., Bd. xlii, 1901, p. 161. *Brinckerhoff* and *Tyzzer:* Jour. of Med. Research, N. S., vol. iii, 1902, p. 449. *Roger* et *Josué:* La Moelle osseuse, etc., L'Œuvre médico-chirurgical, 1899, Paris.

[2] *Lobenstine:* Am. J. Med. Sci., vol. cxxviii, 1904, p. 281.

## PATHOLOGICAL LEUCOCYTOSES

### POLYNUCLEAR NEUTROPHILE LEUCOCYTOSES

These may be divided as follows:

**Leucocytosis in Infectious Diseases.**—The chief diseases in which a well-marked leucocytosis appears are: cholera, scarlet fever, smallpox, erysipelas, diphtheria, pneumonia, acute articular rheumatism, tuberculous meningitis, suppurative conditions in the subcutaneous tissues, the serous cavities, the bones and joints, or the viscera. Leucocytosis is occasionally present in amœbic abscess of the liver though not a regular occurrence. If an abscess in any portion of the body is well encapsulated, or if the infection is a very virulent one, there may be no leucocytosis.

In the following infectious diseases there is either no leucocytosis or a very slight rise: typhoid, measles, rötheln, mumps, malaria, uncomplicated tuberculosis, except when invading the meninges or serous surfaces. In phthisis with a marked sepsis there may be an increase in the number of leucocytes, due to the mixed infection.

**Leucocytosis following the Use of Drugs, etc.**—During and after chloroform or ether inhalations, a moderate increase in the number of leucocytes is seen. Drugs such as quinine, the salicylates, tuberculin, will increase the leucocytes, as will also saline infusions and the inhalation of illuminating gas. The same is true of drugs of the phenacetin type and potassium chlorate, large doses of which cause methæmoglobinæmia and destruction of red cells.

**Leucocytosis following Hæmorrhage.**—The increase is often quite marked within a short time after the loss of blood, only to disappear in a few days as the blood is regenerated.

Under this heading may be considered an increase in the number of leucocytes which frequently exists after a surgical operation. The polynuclear neutrophiles are the cells chiefly concerned in the increase. Myelocytes in small numbers have been seen. The leucocytosis reaches its maximum about twelve hours after the operation and rapidly decreases to normal in twenty-four to seventy-two hours. If the leucocytes continue high an infection is probable.

A portion of the leucocytosis observed under these conditions is undoubtedly due to the ether or chloroform used as an anæsthetic, but as the increase may also be seen after an operation without anæsthesia, a portion of the rise in numbers must be due to the shock of the operative interference.[1]

**Leucocytosis in Connection with Malignant Growths.**—An increase is fairly constantly present in over 50 per cent. of the cases, the phenomenon seeming to depend a good deal on the nature and situation of the tumor. In carcinoma of the stomach, with or without leucocytosis, a digestive increase in the white cells is often absent.

The leucocytes in all these forms of leucocytosis are, as stated above, chiefly of the polynuclear neutrophile variety, but certain variations are often to be noted. The neutrophile cells are not always quite the same as those seen in normal blood;[2] the nuclei approach more nearly to the mononuclear type, and the granulations when stained by means of the Jenner stain or with methylene azure and eosin, are often rather few in number and show the basophilic tendency characteristic of young cells just produced in the marrow.

### POLYNUCLEAR EOSINOPHILE LEUCOCYTOSIS[3]

A marked increase in the eosinophile cells is always pathological and is less commonly seen than in the neutrophile, while the numerical increase in the cells is not usually great—indeed, the leucocytosis is often relative. The normal number of eosinophiles in the blood in health is from 2 to 4 per cent. of the white cells, while in extreme cases of eosinophile leucocytosis the number may rise to at least 90 per cent. of the cells present. The absolute number can be determined by counting with a diluting fluid colored with eosin, and varies under normal conditions from fifty to two hundred and fifty in the cubic millimeter. In a simple eosinophile leucocytosis the number rarely exceeds five thousand, in leukæmia, thirty thousand, while in a case of lymphosarcoma, sixty thousand have been found to the cubic millimeter. The common types of eosinophile leucocytosis are seen in:

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[1] An excellent paper on this subject is by *King:* Am. Jour. Med. Sci., 1902, vol. cxxiv, p. 450.

[2] *Arneth* (Die Neutrophilen weissen Blutkörperchen bei Infektions-Krankheiten, Jena, 1904) in an exceedingly prolix and obscure monograph reports the results obtained by classifying the polynuclears according to the number and arrangement of the nuclear fragments and claims to have obtained facts of diagnostic value by this procedure. Further work on the subject is necessary, however, before the results can be accepted.

[3] The best monograph on the subject is *Zappert:* Zeit. f. klin. Med., Bd. xxiii, 1893, p. 227. See also *Bettmann:* Volkmann's klin. Vorträge, Ninth Series, No. 266, 1900; and *Opie:* American Journal of Med. Sciences, vol. cxxvii, 1904, p. 217.

(*a*) Bronchial asthma, in which the cells are from 10 to 30 or even 50 per cent.[1] of the whole number of whites and are also abundant in the sputum. They gradually disappear after the attacks have ceased for a few weeks.

(*b*) Skin diseases, such as zoster, pemphigus, prurigo, dermatitis herpetiformis,[2] and psoriasis, when of considerable extent, and, rarely, in urticaria. An increase in the eosinophiles to 10 per cent. has been seen by the writer in a case of scleroderma and also in a number of cases of mercurial dermatitis, while 14 per cent. have been seen in a vesicular eruption due to potassium iodide. The blister fluid obtained by applying cantharides to the skin may contain a considerable proportion of eosinophiles. A moderate eosinophilia[3] may be met with in the blood of leprous patients, even as high as 60 per cent., though this is rare. A case seen by the writer showed 17 per cent. during an acute exacerbation of the skin lesions.

(*c*) Intestinal parasites. Eosinophilia has been observed in persons infected with Anchylostoma duodenale, occasionally with Oxyuris, Ascaris, and the Tænias, and recently in those infected with Bilharzia.[4] The number may reach 75 per cent. This rise in the number of eosinophiles has not been noted after prolonged infection with certain species of worms, such as the Bothriocephalus latus,[5] which have incited a severe anæmia; and Bloch[6] reports two cases of pernicious anæmia infected with Tænia solium in which there was an actual diminution of the eosinophiles to about 1 per cent.

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[1] *Billings:* N. Y. Med. Jour., 1897, vol. lxv, p. 691.

[2] *Leredde:* Monatsh. f. Derm., 1898, Bd. xxvii, p. 381.

[3] *Gaucher* and *Bensaude:* Annal. d. Derm., 1896, p. 204.

[4] *Kautsky Bey:* Zeit. f. klin. Med., 1904, Bd. lii, p. 192. *Douglas* and *Hardy:* Lancet, 1903, p. 1009.

[5] *Schauman:* Bothriocephalus Anämie, Berlin, 1894. See also Samm. klin. Vorträge, Tenth Series, No. 287, 1900, p. 273.

[6] Deut. med. Woch., 1903, p. 511.

(*d*) Trichinosis[1] shows a very considerable increase in the eosinophiles in most of the observed cases, even as high as 86 per cent.;[2] but Rosenburger[3] reports a case in which the eosinophiles were only 4 per cent., while DaCosta[4] calls attention to one and Drake[5] to four similar cases. In filariasis[6] also there may be a very considerable increase in the eosinophiles; but the writer has seen one case in which there was no increase. It seems probable that after long-continued infections the blood ceases to respond to the chemotactic substance given off by the parasite.

(*e*) Malignant tumors may be accompanied by an increase of the eosinophiles, a phenomenon which has been observed to be especially well marked in tumors involving the bone marrow by metastasis.

(*f*) Postfebrile eosinophilia reaching only to a moderate degree has been noted to follow acute infectious diseases, especially those with a marked neutrophile leucocytosis during the course of the disease.

(*g*) Toxic eosinophilia has been observed after injections of tuberculin or cinnamic acid and the administration of camphor.

(*h*) Hydatid disease is occasionally accompanied by an eosinophilia, but not constantly.[7]

(*i*) An increase in the eosinophile cells has occasionally been noted in gonorrhœa, especially when the process has extended to the deep urethra and involved the epididymis. In mild cases of anterior urethritis, the leucocytic formula remains normal.

(*j*) A moderate eosinophilia is present in scarlet fever, in contrast to measles, in which no such phenomenon is to be observed.[8]

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[1] *Brown:* Jour. Exp. Med., 1898, vol. iii, p. 315; *Schleip:* Deut. Arch. f. klin. Med., 1904, Bd. lxxx, p. 1.

[2] *Kerr:* Phila. Med. Jour., 1900, p. 346. *Blumer* and *Neuman:* Amer. Jour. Med. Sci., 1900, vol. cxix, p. 14.

[3] Proceedings of Path. Soc. of Phila., vol. iv, No. 10.

[4] Clinical Hæmatology, Phila., 1902. See also *Cutler:* Trans. Ass'n. American Physicians, 1902, p. 356.

[5] Jour. Med. Research, N. S., 1902, vol. iii, p. 255.

[6] *Calvert:* Johns Hopkins Hosp. Bull., 1902, p. 133. *Dudgeon* and *Child.* Journal of Tropical Medicine, 1903, vol. vi, p. 253.

[7] *Bloch:* Deut. med. Woch., 1903, p. 511. See also *Gouraud:* Bull. Soc. Anatomique d. Paris, 1902, for a negative case.

[8] *Reckzeh:* Deut. Arch. f. klin. Med., 1903, Bd. lxxvii, p. 330. (Bibliography.)

### BASOPHILE LEUCOCYTOSIS

Basophile leucocytosis is practically seen only in myelogenous leukæmia, in which disease these cells may be present in very large numbers. In one specimen of myelogenous leukæmia in the possession of the writer, the total leucocyte count was one million two hundred thousand, the basophiles forming from 10 to 15 per cent. of the white cells, or one hundred and twenty thousand to one hundred and eighty thousand to the cubic millimeter, varying from time to time as the disease progressed. Taylor also reports a case in which the basophiles numbered one hundred and forty thousand to the cubic millimeter.

Under ordinary conditions the basophiles present in the blood are not over $\frac{1}{2}$ of 1 per cent., but after a sharp hæmorrhage they may rise to 1 or even to 3 per cent. Zollikofer[1] reports a moderate basophile leucocytosis in an hysterical patient during an attack of sciatica, and in another case these cells were increased during a non-febrile articular rheumatism. Taylor[2] saw an increase in a case of carcinoma with cachexia but without bone-marrow metastases; also in a case of gonorrhœa with basophile cells in the discharge; one of mycosis fungoides, and two cases of septic bone disease. Canon[3] also noted an increase in cases of chronic skin disease. An increase in the basophile cells in the blood of the rabbit can be easily produced by the injection of bacterial toxins, but the rise follows that of the other cells of the blood, notably the polynuclears, while in most of the leucocytoses seen in the human subject, as in pneumonia, for example, there is no increase in the basophile cells. In ascitic fluids obtained from persons the subjects of myelogenous leukæmia, Milchner[4] has found 33 per cent. of mast cells, and 5 per cent. of mast cells has been found in fluid from a cantharides blister in a case of the same disease. Basophile cells have been found in pleural exudates without an increase in the same cells in the blood.[5] Such a phenomenon must be the result of active emigration from the blood in response to a positive chemotactic stimulus.

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[1] Diss. Berne, 1899.

[2] Cont. from Pepper Laboratory, 1900, p. 267.

[3] Deut. med. Woch., 1892, p. 206.

[4] Zeit. f. klin. Med., 1899, vol. xxxvii, p. 194.

[5] *Wolff:* Münch. med. Woch., 1902, p. 226. *Bibergeil:* Cent. f. d. med. Wissen., 1904, p. 338.

### LYMPHOCYTOSIS

A marked absolute lymphocytosis is very rarely seen in any disease except lymphatic leukæmia. A moderate relative lymphocytosis is present in the latter part of well-marked cases of typhoid fever, and in children with hereditary syphilis or intestinal diseases of an acute nature. In pertussis a marked leucocytosis is often present, and a relative and absolute increase in the lymphocytes may occur even to as high as 20,000 to the cubic mm. In pernicious anæmia also, the lymphocytes are relatively increased, although a marked leucopenia exists. The same change is occasionally noted in hepatic cirrhosis, especially when a marked anæmia is present.

### LARGE MONONUCLEAR LEUCOCYTOSIS

The increase is usually relative. These cells are increased in malarial cachexia and may act as phagocytes in that disease. They are also increased in carcinoma in the cachectic stage and in measles. At times they are said to form a considerable per cent. of the leucocytes in myelogenous leukæmia, though they are to be carefully distinguished from the early non-granular type of myelocytes or myeloblasts seen in very advanced cases. No doubt in many cases of leukæmia in which the mononuclears have been considered as increased, this distinction has not been observed.

### MYELOCYTOSIS

The appearance of myelocytes in considerable numbers is most commonly noted in connection with myelogenous leukæmia where all varieties of this form of cell may appear in the peripheral blood; but they may occasionally be seen in acute lymphatic leukæmias where marked hyperplasia of the bone marrow exists. In pernicious anæmia a number of myelocytes are not uncommonly present in the blood. They also appear in the blood of severe secondary anæmias, in multiple myeloma and especially in connection with malignant disease, with or without involvement of the bone marrow. In the latter case they are especially abundant. They may be noted in the anæmia dependent upon syphilis and tuberculosis. In acute infections they are most often seen in connection with high polynuclear leucocytoses, especially in diseases in which the marrow is more or less affected, as in variola, in empyema in children, and in diphtheria.

Taylor has noted them in connection with an acute case of tertian malaria. Schwyzer[1] records a case of chronic fluorine poisoning in which myelocytes were present in considerable numbers in the blood. Their appearance under these circumstances is probably the result of the increased activity of the marrow and depends either upon mechanical conditions due to bone changes produced by the poison or upon a toxic chemotaxis.

### LEUCOCYTOSIS IN CHILDREN

Leucocytosis in children shows certain variations from that in adults, especially in the proportion of the mono- to the polynuclear cells. As is well known, the relationship in normal blood between the two forms in adult life is as about one to three, while in children under one year of age the relationship is often inverted, so as to be two to one.

In pneumonia the polynuclears rise very high, up to 90 per cent., while the eosinophiles practically disappear, to reappear in increased numbers after the crisis. Myelocytes may appear in cases of pneumonia, empyema, and diphtheria, with a very severe course, and occasionally nucleated red cells are seen after a prolonged infection. There is a leucocytosis in hereditary syphilis, but the polynuclears remain low, often not over 20 per cent., with great increase of the lymphocytes, and there may also be an occasional marked increase in the eosinophiles. In general, it may be said that the blood in children is much more sensitive to stimuli than that of adults, as is well shown by the digestive leucocytoses in infants, which may rise to thirty-five thousand just after a meal.

## V. SPECIAL PATHOLOGY OF THE BLOOD

### POLYCYTHÆMIA

This term is used to designate certain conditions in which the number of corpuscles in the blood is increased without regard to the amount of hæmoglobin in the individual corpuscle, though, as a rule, this amount is approximately normal. The term, therefore, is not the converse of anæmia.

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[1] Journal of Medical Research, 1903, N. S., vol. v, p. 301.

**Physiological Polycythæmia.**—This increase in the number of red cells in the blood is normally found in new-born infants, a fuller discussion of the subject being found on page 108. A marked polycythæmia is found in the blood of persons residing at a considerable elevation above the sea level, where the counts may rise to six, seven, or even eight million corpuscles to the cubic millimeter. This change is not permanent and there is a gradual diminution in the number of corpuscles after two or three years' residence, to a point usually a trifle higher than the count on the same person at sea level. Permanent residents in high altitudes usually show counts slightly over the sea level normal of five million.

The conditions underlying this change are not wholly understood. By many it is considered to be a compensatory hyperplasia due to the lowered oxygen tension of the atmosphere. Others have suggested that the change is due to concentration of the blood owing to the increased evaporation from the skin and the stimulus of the rarefied air. On returning to sea level the number of corpuscles falls to the average of five million. The high counts are not due to errors in the hæmocytometer chamber brought about by the lowered atmospheric pressure, as was originally thought.[1]

**Pathological Polycythæmia.**—An increase in the number of corpuscles is seen after severe watery diarrhœa, especially in Asiatic cholera, when though the total number of corpuscles in the body may be diminished, yet the removal of large quantities of fluid produces an extreme concentration of the blood. In the same way a rapidly forming exudate, profuse sweating, persistent vomiting, and starvation, may remove water from the blood, and thus apparently increase the number of corpuscles. Occasionally we see a case of carcinoma of the pylorus in which persistent vomiting has continued for a few days, and although a marked anæmia would naturally be expected from the starvation resulting from the obstruction and the toxic effect of the new growth, yet a mere enumeration of the corpuscles may show nearly normal values. The hæmoglobin, however, may be lowered owing to the anæmia of the individual red cell.

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[1] For a general review of this subject, see *Kronecker:* Die Bergkrankheit, Berlin, 1903. See also *Kemp:* Proc. Am. Phys. Soc., Amer. Jour. of Phys., vol. x, 1904, p. xxxii.

Polycythæmia has also been noted in jaundice and in connection with poisoning by phosphorus and by carbonic oxide, though the underlying causes are not as yet understood.

Polycythæmia is also met with in congenital[1] or acquired heart disease, the counts in persons with congenital lesions often rising to six or seven millions. Considerable differences have been observed in the counts of the blood from a vein and from the finger tip, showing that in uncompensated heart disease there may be an irregular distribution of the corpuscles. The increase in the number is probably due to a compensatory hyperplasia of the red corpuscles to make up for the imperfect aeration of the tissues by the venous blood which circulates through them.

The writer has frequently noted in persons suffering from broken compensation due to either valvular or muscular lesions of the heart, a considerable number of normoblasts, in one case rising as high as 5,000 to the cubic millimeter. Polychromatophilia and granular degeneration are also frequently present. All of these morphological changes in the red cells disappear promptly either on the continuous administration of oxygen, or as compensation is obtained from rest in bed, or by the action of drugs. The rapid alterations in the number of cells which may be observed in persons with either congenital or acquired heart disease show, however, that a hyperplasia of the cells is not the only factor. A portion of the polycythæmia must be due to some concentration of the corpuscles in the vessels of the periphery. Polycythæmia also accompanies cyanosis produced by any interference with the aeration of the blood of the lungs. It has been noted in stenosis of the larynx and in pneumothorax. Some cases of chronic pulmonary tuberculosis with large cavities show high or normal counts with very low hæmoglobin. In persons suffering from marked chronic pulmonary emphysema the figures for the red cells are apt to be high. The same condition is found in persons suffering from bronchiectatic cavities in the lung of a nontuberculous nature. Such cases often show marked clubbing of the fingers and other evidences of imperfect circulation.

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[1] *Wile:* Proceedings of the New York Path. Society, N. S., vol. iv, p. 36. *Grawitz:* Deut. Arch. f. klin. Med., 1895, Bd. liv, p. 588.

Polycythæmia has also been met with in cases of enlargement of the spleen with chronic cyanosis, a few examples of which have recently been described in the literature.[1]

## ANÆMIA

The expression anæmia denotes a deterioration in the quality of the blood which may affect either the red cells or the hæmoglobin or both. These changes in the composition of the blood make themselves manifest by a number of symptoms, among which the most striking are pallor of the skin and mucous membranes. This pallor may be an accurate index of the degree of impoverishment of the blood, or may indicate only an abnormally small capillary circulation in the corium. Dwellers in tropical countries often show the most marked pallor with a normal blood count, while a blond of the Teutonic race may have a high color with a considerable degree of anæmia. The mucous membrane of the palpebral conjunctiva or of the lips, or the amount of vascularization seen under the nails, is much more likely to afford reliable evidence of the presence of an anæmia than the skin.

Freshly drawn blood from all varieties of anæmia shows certain aberrations from the normal, such as low specific gravity, less depth of color as it flows from the puncture, and a diminution in rouleau formation, which permits the experienced observer to draw conclusions of diagnostic value; but, however important such facts may be, they can never replace a careful and systematic study of both fresh and stained preparations.

The anæmias may be classified most simply under three divisions of (1) chlorosis, (2) secondary anæmia, and (3) pernicious anæmia. It must be remembered, however, that each of these anæmias can not be promptly relegated to its respective class by a simple examination of a smear of blood; on the contrary, each of these so-called types is artificial and arbitrary, and the distinctions which are made between the various types are based upon an average morphology, so to speak, and each group may have clustered about it a large number of types which differ only slightly from each other and gradually bridge over the sharp distinctions between the groups. In other words, if we should arrange the anæmias in a tabular form, beginning with that which shows the least blood change in the average case and ending with that which shows the most, we should begin with chlorosis and end with pernicious anæmia. Between the two ends of the series the difference in the blood morphology is so striking that there is no possibility of confusion, but to distinguish between the forms lying adjacent to one another is at times very difficult, if not impossible, merely from examination of the blood alone.

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[1] *Rendu* et *Widal:* Soc. méd. des hôpitaux, 1899, p. 528. *Türk:* Wien. klin. Woch., 1902, p. 372. *Osler:* Trans. of the Assoc. of Am. Physicians, 1903, vol. xviii, p. 299.

The scheme below is merely a diagram to indicate roughly the conditions met with in classifying the anæmias and to indicate also the intimate relationship which exists between the types. Beginning with the simpler anæmias, like the chloroses, which show red cells of practically normal dimensions and numbers in mild cases, there is a gradual transition to the pernicious form in which the red cells show the most extreme alterations. The differences between the members of the series are qualitative and not absolute, for even in chlorosis, the mildest of the simple forms, it is possible very rarely to find megaloblasts in advanced cases, while in pernicious anæmia, they, as a rule, form the greater proportion of the nucleated red cells, even in the less developed forms. So, too, an anæmia due to the cestode worm, Bothriocephalus, may show on examination only a mild anæmia of the

| | |
|---|---|
| Normoblastic and microcytic type. | Chlorosis |
| ↑ | ↕ |
| | Secondary anæmia due to bad hygienic conditions. |
| | ↕ |
| | Secondary anæmia due to general disease. |
| ↓ | ↕ |
| Intermediate type, may be macro- or microcytic. | Secondary anæmia due to poisoning (lead, etc.). |
| ↑ | ↕ |
| | Secondary anæmia due to tumors, chronic hæmorrhage, etc. |
| | ↕ |
| | Secondary anæmia due to intestinal parasites. |
| ↓ | ↕ |
| Megaloblastic and macrocytic type. | Pernicious anæmia (cryptogenetic anæmia). |

chlorotic type, while in other cases the anæmia produced by this worm can not be distinguished from pernicious anæmia by the blood examination, but only by finding in the stools the characteristic eggs of the worm.

The acute anæmia following hæmorrhage is not included in the scheme as it belongs to a special class, the changes present being dependent upon the rapid loss of blood and the effort of the bone marrow to make up for the deficiency as quickly as possible.

### ANÆMIA FOLLOWING HÆMORRHAGE

The lowering of the number of the red cells and the hæmoglobin is roughly proportional to the amount of blood lost, but there is always a slight continuous and progressive reduction during the first twenty-four hours following the hæmorrhage. This is due to the fact that it takes a certain time for the tissue fluids to transude into the vessels to replace the loss of blood, and this transudation makes itself felt for some time in a slow reduction of the red cell count. Occasionally there is a slight fall lasting for some days after the hæmorrhage, which may be due to the lessened resistance of the corpuscles first turned out by the bone marrow to replace the loss.

The amount of blood which may be lost, as evidenced by the reduction in the number of red cells, depends largely upon the rapidity with which the blood is removed from the vessels, as it is evident that a much larger amount may be lost by a gradual oozing than by a sudden removal of a large quantity.

The reduction of the red cells to nine hundred thousand to the cubic millimeter has been seen in a hæmorrhage extending over ten hours' time, and Hayem reports a case in which the diminution of the red cells reached to five hundred and fifty thousand in a hæmorrhage extending over six days' time. The restitution of the lost cells may begin within a day or so, but is occasionally delayed for eight to ten days, depending upon the amount lost and the general health of the patient before the hæmorrhage. The time required for complete recovery is also dependent upon the amount, and it may take several months before the normal number of cells is found in the blood. Bierfreund[1] states that regeneration may be complete in three weeks, if the blood lost does not exceed 20 per cent. of the whole, or four weeks after the loss of 25 per cent. The hæmoglobin in this regeneration does not follow the upward rise of the red cells, but lags considerably behind. This may be due to two causes —the newly formed cells as they leave the bone marrow may not contain their normal amount of hæmoglobin, or the regenerated cells may be microcytic, thus giving a high numerical count with a low hæmoglobin percentage. Both factors usually obtain. Tallqvist[1] has found that the amount of iron present in the liver has an important bearing on the rapidity of replacement of the hæmoglobin, a large stock of "reserve" iron conducing to quick recovery. The specific gravity of the blood is reduced to a considerable degree, proportional to the amount of blood lost.

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[1] Langenbeck's Arch., 1890, Bd. xli, p. 1.

The morphological changes in the red cells are usually well marked and reach a very extraordinary extent in slow, long-continued hæmorrhages, owing to the degenerative, or, perhaps more accurately, regenerative changes which take place in the blood-producing organs.

After every severe loss of blood, careful search will reveal the presence of normoblasts in small numbers; occasionally they may form a considerable proportion of the red cells. Their appearance in the peripheral blood is not continuous, but in the form of the so-called "blood crises."[2] That is, they appear at irregular intervals in large numbers and are usually present for a few days only, while between the "crises" the blood may be comparatively free. These crises have also been noted during the treatment of anæmias with arsenic.[3] This same intermittent action of the bone marrow is apparent in other diseases and also in the production of the white cells. The appearance of normoblasts in the blood is an evidence of the inability of the bone marrow to cope with the sudden demand for a large number of new cells, and their disappearance an indication that the normal blood formation is again taking place. Rarely microblasts are seen, and during the recovery from a very great and long-continued hæmorrhage an occasional megaloblast may appear. Within a short time after the hæmorrhage a moderate polychromatophilia is often apparent, which, however, soon disappears unless the hæmorrhages are repeated. Poikilocytes are frequently seen and also microcytes.

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[1] Exp. Blutgift Anämien, Helsingfors, 1900.

[2] *v. Noorden:* Charité Annalen, Bd. xvi, 1891, p. 217.

[3] *Askanazy:* Zeit. f. klin. Med., Bd. xxvii, 1895, p. 503.

The red cells for a few days may show a distinct increase in bulk for the individual cell, which is best made evident by determining the bulk by the centrifuge, though it may be shown by microscopical examination of the individual corpuscles. This change is thought to be due to the lowering of the osmotic tension of the serum by the transudation of the tissue fluids into the blood and the absorption of the water by the red cells. The loss of

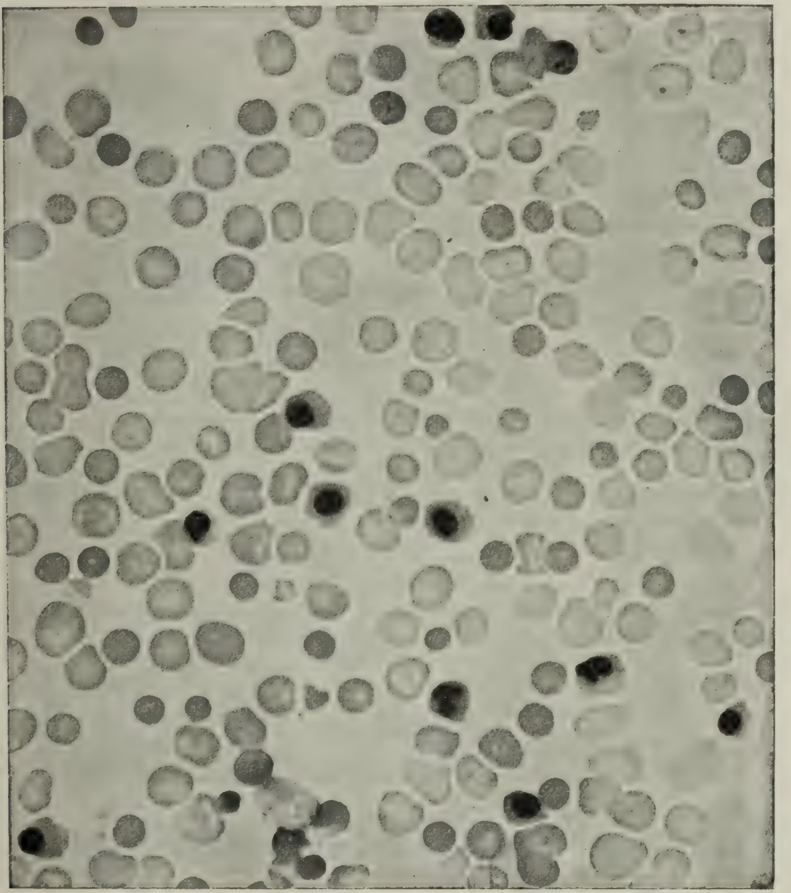


Fig. 35.—Normoblastic Crisis in a Case of Severe Anæmia. Magnified 700 diameters.

# DESCRIPTION OF PLATE IV

*Stained by the Jenner Method*

### *Figure A.*—NORMAL BLOOD

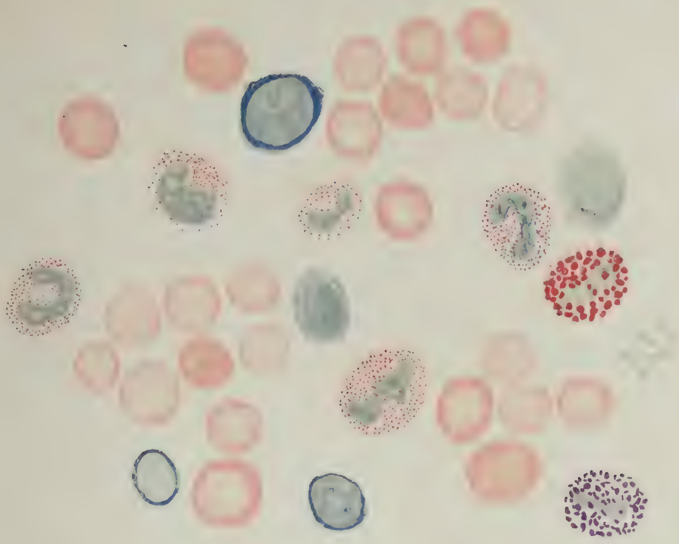
The red corpuscles differ slightly in size and shape, and some of them are more deeply stained in the center than others, variations which are constantly met with in normal blood. The leucocytes in the drawing are more abundant in relation to the red cells than normal in order to include a sufficient number of them in a single field of the microscope.

Five polynuclear neutrophile leucocytes are present in the figure, showing the common variations in the form of the nuclei. The cell in the upper left corner corresponds to the transitional type of neutrophile leucocyte. In the upper portion of the field is a large lymphocyte with a deeply stained cell body which shows two small projecting masses. Below are two small lymphocytes, one of which shows nucleoli. To the right from above downward are a large mononuclear leucocyte, an eosinophile leucocyte, a small group of blood plates, and a basophile cell (mast cell.) In the center of the figure is a medium-sized lymphocyte with a faintly staining cell body and dark blue nucleus. This variant from the usual stain is especially abundant when the Jenner preparation has been washed with tap water, and may also be seen in the lymphatic leukæmias. A complete series can usually be made out between the lymphocytes and the large mononuclears.

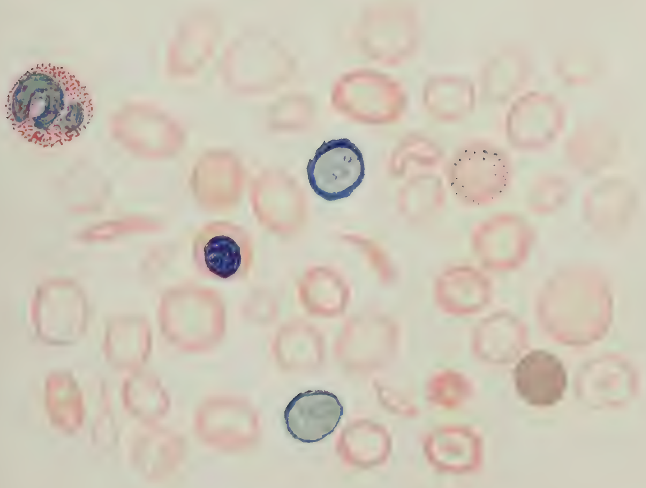
### *Figure B.*—CHLOROSIS

The changes of importance in chlorotic blood are practically confined to the red cells. The erythrocytes show marked variations in shape (poikilocytosis) and in size (anisocytosis). The average diameter is, as a rule, slightly below that of normal blood, though occasionally very large corpuscles may be seen. The central depressions are much more extensive than in normal blood, many of the cells showing only narrow rings of stained protoplasm.

Crenation is much more apt to occur in chlorotic than in normal blood. Granular degeneration will be noted in a cell above to the right, while below is a purplish red cell showing polychromatophilia. A normoblast is figured slightly to the left of the center. The cell body is orthochromatic, the nucleus quite compact and deeply staining (pycnotic). The leucocytes are the same as in normal blood.



A.—NORMAL BLOOD.



B.—CHLOROSIS.

PLATE IV.—NORMAL BLOOD AND CHLOROSIS.

hæmoglobin in the cells is often apparent from their low staining capacity and the increased size of the central depression.

The white cells are, as a rule, increased in numbers, which is usually due to an absolute increase in the number of polynuclear neutrophiles, though rarely there may be an increase in the number of the lymphocytes. The basophiles may rise as high as 3 per cent. shortly after the hæmorrhage. Sometimes a moderate number of myelocytes may appear in the blood; in one case of Ehrlich's they formed 14 per cent. of the white cells, but such a phenomenon is usually quite transitory and only an evidence of an extreme stimulus to the bone marrow, which causes it to turn out imperfectly matured cells. The bone marrow of children is much more likely to act in this manner than that of adults.

This stimulus to the bone marrow results in a marked hyperplasia of this structure and the replacement of the normal fatty marrow in the shafts of the long bones with red marrow, consisting largely of nucleated red cells. The degree of this replacement is dependent upon the amount and length of time over which the loss of blood extends; in acute hæmorrhages with a single large loss of blood the hyperplasia is usually not great. The coagulability of the blood and the number of blood plates are increased after a hæmorrhage.[1]

## CHLOROSIS

The blood changes in chlorosis upon which emphasis should be laid are, first and most important, a relatively high red cell count with a low hæmoglobin per cent. The red cells may remain at the normal for women (4,500,000) or they may fall to between 1,500,000 and 2,000,000, but in any case the hæmoglobin value of the red cells is greatly reduced, often to 20 or 30 per cent. by Fleischl, and averaging 40 per cent. in a large series of cases. The color index in three hundred and fifty cases of chlorosis averaged about 0.5. This relatively low index is most marked in first attacks in young women; in recurrences it is not so striking, nor is it so evident in the chloroses of older persons or in long-continued anæmias. The red cell's average diameter in chlorosis is unchanged or diminished, but individual corpuscles vary a good deal (see Fig. 36). Occasionally one sees megalocytes measuring 12 to 15 micra, but small cells are much more abundant. The red cells when stained lightly with eosin show a very marked increase in size of the pale central depression, especially in those cells which lie in the thicker part of the smears. The change is not well marked in Jenner preparations. Even the poikilocytes may be mere faint rings of stained protoplasm. The latter are absent in the light forms, more marked in the severe forms of the disease. They may disappear from the blood in a week with suitable treatment.

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[1] For an experimental study of the blood changes after hæmorrhage, see *Willebrand:* Zur. Kennt. d. Blutveränd. nach Aderlässen, Berlin, 1900.

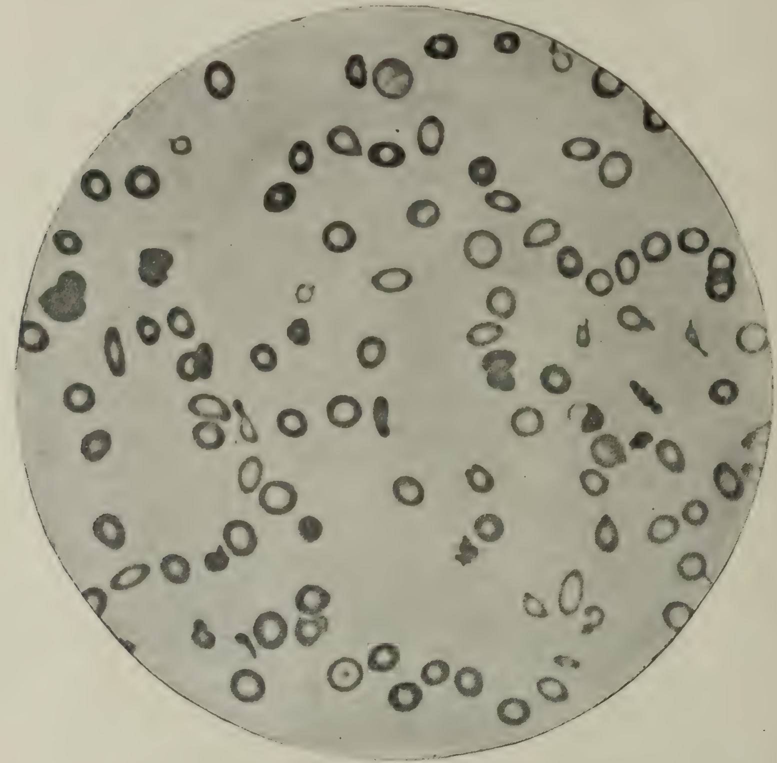
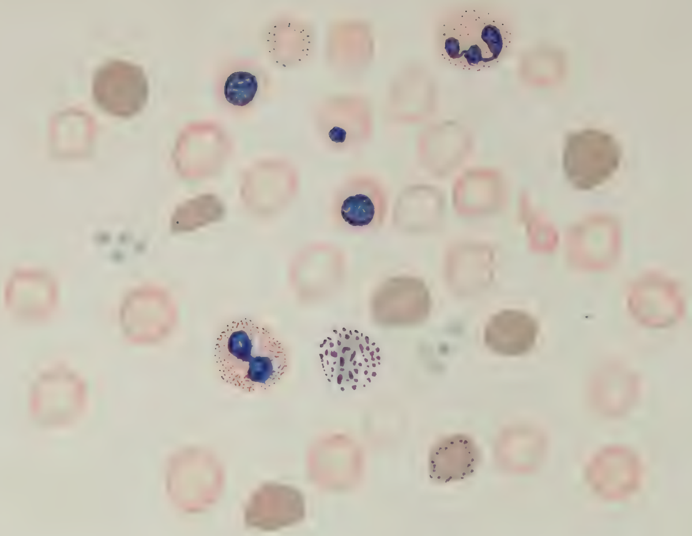
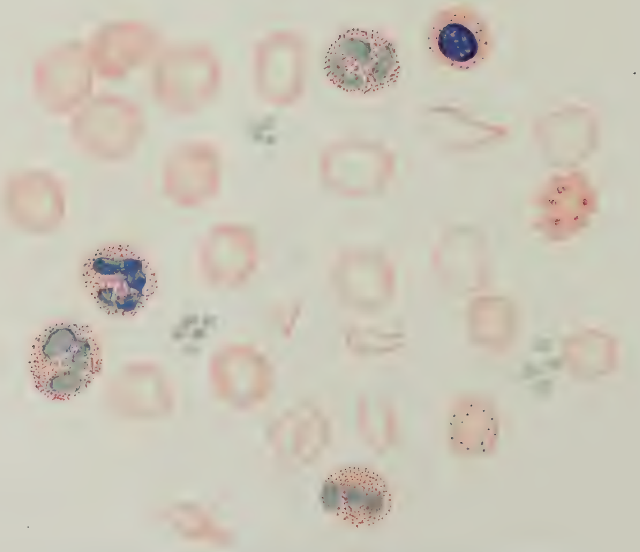


FIG. 36.—CHLORÓTIC BLOOD. Magnified 700 diameters.

Normoblasts can be found, after careful search, in all well-marked cases. Megaloblasts have been seen, but with extreme rarity and only in very advanced anæmia. Polychromatophilia



A.—SECONDARY ANÆMIA FROM HÆMORRHAGE.



B.—SECONDARY ANÆMIA FROM CARCINOMA.

PLATE V.—SECONDARY ANÆMIA.

# DESCRIPTION OF PLATE V

*Stained by the Jenner Method*

*Figure A.*—SECONDARY ANÆMIA FOLLOWING HÆMORRHAGE

The changes in the red cells are much the same as in chlorotic blood, with the exception that erythrocytes showing granular degeneration and polychromatophilia are usually more abundant owing to the rapid regeneration of the red corpuscles, and normoblasts are apt to be present in considerable numbers.

The leucocytes are usually increased and the basophile cells are much more numerous than in normal blood. The red cells in the upper portion of the field show the usual arrangement of the granules in punctate degeneration; that is, they are arranged in a broad ring at the periphery of the corpuscle. Three normoblasts are shown, two of which have large nuclei with suggestions of a chromatin network; the third has a small pycnotic nucleus. In the lower right-hand corner of the field is a red cell containing a central mass of hæmoglobin. This picture is frequently met with in the anæmias and is of no diagnostic significance.

*Figure B.*—SECONDARY ANÆMIA FOLLOWING CARCINOMA OF THE STOMACH

The changes in the red cells are chiefly a loss of hæmoglobin, as evidenced by the marked central depression. A moderate poikilocytosis is present. A normoblast is shown in the upper portion of the field whose cell body is filled with basophilic granules. Cells of this type are especially abundant in myelogenous leukæmia, but are occasionally seen in the secondary anæmias. The leucocytes are chiefly those of the polynuclear neutrophile variety.

and granular degeneration may be present in moderate amount. The leucocytes remain normal in number; but there is usually a slight relative lymphocytosis and a marked reduction in the eosinophiles. A typical differential count is: small lymphocytes, 18 per cent., large lymphocytes, 17 per cent., polynuclears, 64 per cent., eosinophiles, 1 per cent.

Myelocytes have been seen very rarely. The specific gravity of the whole blood is lowered in proportion to the hæmoglobin reduction. The iron content of the blood varies roughly with the hæmoglobin. The serum remains quite constant in its composition. It must always be remembered that the diagnosis of chlorosis can not be made from a stained slide nor from a blood count and hæmoglobin estimation alone. The whole clinical picture must be taken into consideration. In other words, a mild chlorotic change in women may be produced in many ways, and a mere blood examination may not furnish absolute points for a differential diagnosis.

### SECONDARY ANÆMIA DUE TO BAD HYGIENIC CONDITIONS

This type of anæmia is one which is frequently met with in hospital and dispensary practice, furnishing the largest proportion of the simple anæmias encountered under these conditions. The most active agent in the production of this form of anæmia is probably not so much bad air and lack of light, as is usually assumed, but the lack of food containing a sufficient amount of iron to supply the body waste. This lack of iron is mainly owing to the small amount of meat consumed by the poor because of its high cost. The same condition is active in regard to the vegetables which contain iron in sufficient quantity, so that the diet of the poor is often largely farinaceous and contains but little iron. The effects of this limitation in the essential constituent of the hæmoglobin are often of considerable extent and affect chiefly the hæmoglobin content of the red cells, but at times reduce the number of the latter. At times the most extreme anæmia is seen, for which the only apparent cause is an extreme degree of malnutrition determined solely by the conditions outlined above. Von Limbeck reports a case in which the red cells were reduced to 306,000, yet in a few months of treatment they reached 4,280,000. The specific gravity is low, corresponding to the reduction in hæmoglobin. A leucocytosis is not seen nor are the proportions between the leucocytes altered to any marked extent.

### SECONDARY ANÆMIA DUE TO GENERAL DISEASE

**Prolonged suppuration** is capable of causing a marked reduction in the red cells and the hæmoglobin of the blood by a combination of conditions, the most important of which are the direct action of the toxins of the bacteria on the blood and the bone marrow by a destruction of the first and a diminution of the blood-producing power of the second. A minor factor is the loss of serum which takes place in the exudate and the inability of the patient to replace the loss because of the faulty digestion and assimilation which exists, due to the septic condition of the case. A good example of this type of anæmia is the marked and rapidly increasing impoverishment of the blood seen in puerperal infections, where the red cells may fall to 20 per cent. of their original number in the course of a few days.

**Acute Infectious Processes.**—Fever, or rather, the conditions which incite fever, is capable of causing very considerable anæmia, especially if the infection be long continued as in typhoid fever. The very constant appearance of an increase of the urobilin in the urine of patients with a febrile condition is an evidence of a greatly increased blood destruction. An interesting example of fever excited by a toxin and accompanied with increase in the excretion of urobilin is seen after the injection of Koch's tuberculin.

**Diseases of the gastro-intestinal system,** either with atrophy or injury of the mucous membrane or with the absorption of toxins from the intestinal contents, are often a determining factor in the production of more or less severe anæmia. This may be especially severe after a protracted enteritis or colitis, especially where there has been a moderate extravasation of blood from the inflamed mucosa, thus adding hæmorrhage to the other causes for blood destruction. The alterations which take place in the absorption and digestion of the food in such cases of gastro-intestinal disorder also add the symptoms due to inanition.

**Syphilis** may incite a moderate anæmia in the first stage of the disease, but that of the second stage is usually much more marked and its degree to a certain extent records the severity of the infection. Anæmias of great severity are occasionally seen during the tertiary stage of syphilis, which at times closely resemble true pernicious anæmia in the morphology of the blood and more especially in the general clinical appearance. The leucocytes in syphilitic anæmia are not increased. Justus[1] claims that in constitutional syphilis the initial doses of mercury cause a rapid fall of from 10 to 20 per cent. in the amount of hæmoglobin and that this phenomenon can be used from a point of view of diagnosis. The method is to give an inunction in an adult of about 4 grams of mercurial ointment after having made a careful preliminary estimation of the hæmoglobin, and then to make another test of the hæmoglobin, twelve to eighteen hours later. These observations have not been generally accepted,[2] and the writer in a number of cases has not been able to note greater fluctuations than occur normally in the hæmoglobin of the blood.

**Malarial fevers** are capable of reducing the number of red corpuscles to an extraordinary degree, numbers as low as 500,000 to the cubic millimeter having been reported in cases of chronic malarial cachexia. The method of destruction in this disease can be easily observed under the microscope by watching the segmentation of one of the mature organisms and noting the mere shell of corpuscular substance remaining. An interesting condition often seen in the red cells in malaria, is a well-marked granular degeneration, while in a case which has existed for some time and produced a moderate anæmia a moderate number of cells with polychromatophilia may be seen. Infection of the red cells by the tertian parasite, or more rarely by the æstivo-autumnal sets up a granulation of the body of the red cells which differs from the ordinary granular degeneration in the fact that the particles assume a reddish stain instead of a blue, when colored with methylene azure. A relative lymphocytosis is often met with due to an increase in the larger forms.

### SECONDARY ANÆMIA DUE TO POISONING

An anæmia of great practical importance is due to poisoning by lead. The condition found in the blood is probably due to several factors; the direct action of the lead compounds on the red cells and the blood-forming organs and the alteration in the mucous membrane of the gastro-intestinal tract being the most important, while the chronic nephritis which so often accompanies chronic lead-poisoning also contributes to the anæmia in a certain number of cases.

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[1] Deut. Arch. f. klin. Med., 1903, Bd. lxxv, p. 1.

[2] *Oppenheim* und *Löwenbach:* Deut. Arch. f. klin. Med., 1901, Bd. lxxi, p. 425; 1903, Bd. lxxv, p. 22.

The action of the lead on the gastro-intestinal tract produces lesions of considerable severity which interfere with the proper digestion and absorption of the food, but this change is difficult to determine clinically: the blood, however, shows morphological alterations which are often quite characteristic. The anæmia produced is of no especial type and does not differ from the varying grades of secondary anæmia produced by other causes, but the changes which take place in the red cells are of great interest, especially as they can be easily reproduced in animals.

The alterations found are of two kinds: granular degeneration and polychromatophilia. The granular degeneration is more abundant in lead-poisoning than in any other form of secondary anæmia, and in a severe case a large proportion of the red cells may be altered. The number of granulated cells varies with the severity of the attack and especially with the severity of the clinical symptom of the colic. When all the granules disappear it is an evidence that the disease is lessening in severity and that attacks of colic are not likely to occur. The polychromatophilia is usually well marked, and such cells often contain small vacuoles and other evidences of degeneration. Normoblasts may be found in advanced cases, and occasionally a few megaloblasts in the most severe forms which usually lead to a fatal termination. Cases have been seen in which the blood became megaloblastic in type and in its general morphology resembled that of pernicious anæmia. The leucocytes are not increased or altered in their proportions to one another.

Arsenic occasionally produces a severe anæmia, but without any special characteristics except for a moderate amount of granular degeneration. Copper, cobalt, thallin,[1] and the inhalation of illuminating gas also give rise to granular degeneration, but under ordinary conditions the phenomenon, if well marked, is diagnostic of lead-poisoning.

Extreme anæmia from poisoning by potassium chlorate[2] and by substances of the aniline group has been observed in a number of cases,[1] one of which was due to nitrobenzol [2] and is of sufficient interest to warrant an abstract.

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[1] *Keil:* Diss. Rostock, 1901.

[2] *Jacob:* Berl. klin. Woch., 1897, p. 580 (potassium chlorate).

After taking about one hundred grains of the poison, the blood was chocolate colored and examination showed the presence of methæmoglobin. The red cells were reduced before death to 900,000 and the nucleated red cells became very abundant, rising to 24,700 to the cubic millimeter. During the early part of the disease a considerable proportion of these cells were normoblasts, but later megaloblasts preponderated. Mitoses were seen in considerable numbers, but more usually the changes in the nuclei were of a degenerative nature. The leucocytes were increased to 61,000 and many myelocytes were present. Polychromatophilia was well marked as was granular degeneration. Many of the red cells were poikilocytic, and there were numerous fragments of the erythrocytes, such as are seen in the artificial anæmias, produced by injecting pyrodin into animals.

An interesting case of chronic poisoning with acetanilid producing similar blood changes is that of Stengel and White.[3] For four or five years the patient had been taking considerable quantities of acetanilid. At the time of the first examination, the blood was found to contain about three million red cells and twenty thousand whites. The urine contained considerable albumin and a few granular casts. The second examination of the blood showed 2,092,000 red corpuscles, 71,400 nucleated cells, and 35 per cent. of hæmoglobin. Thirty-two thousand three hundred and twenty-three of the nucleated cells were red corpuscles, leaving thirty-nine thousand leucocytes. Ninety-one and four-tenths per cent. were normoblasts, many of which showed irregular clover-leaf nuclei. Three and five-tenths per cent. were megaloblasts and 5.3 per cent. were free nuclei. The non-nucleated red corpuscles varied greatly in size, megalocytes and poikilocytes being abundant. Many of the red cells contained mitoses. Granular degeneration and polychromatophilia were present in many cells.

Differential count of the leucocytes showed: polynuclears, 72.4

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[1] *Brown:* Am. Jour. of Med. Sci., vol. cxxii, 1901, p. 770 (acetanilid).

[2] *Ehlich* and *Lindenthal:* Zeit. f. klin. Med., 1896, Bd. xxx, p. 427. *Boas:* Deut. med. Woch., 1897, p. 817 (nitrobenzol).

[3] University of Penn. Medical Bulletin, vol. xv, 1903, p. 462.

per cent.; mononuclears, 5.4 per cent.; transitional, 6.6 per cent.; lymphocytes, 11.4 per cent.; eosinophiles, 0.8 per cent.; myelocytes, 3.4 per cent. After the taking of the drug had been stopped, the blood returned to normal in about six weeks.

### SECONDARY ANÆMIA DUE TO TUMORS

**Benign tumors,** even if they are of considerable size and rapid growth, do not alter the composition of the blood unless some complication arises, such as the pressure on some important organ or the ulceration and breaking down of the tumor itself.

**Malignant tumors,** on the other hand, no matter what their situation may be, cause a distinct reduction in the amount of hæmoglobin of the blood and often a marked reduction in the number of the red cells. The *situation* of the tumor, however, exerts an important influence on the process of production of the anæmia. If it is in such a location that pressure or invasion of an important organ takes place, the amount of anæmia produced is greater than in a tumor of similar size and nature so placed that it does not press upon nor invade important structures.

Especially is this true of tumors involving the bone marrow, which often, though not always, produce a most rapid and severe type of anæmia with the appearance in the blood of numbers of nucleated red cells and degenerate forms. *The type of tumor* seems also to exert an influence on the composition of the blood, especially if metastases are formed early. An example of such influence is seen in the slight anæmia produced by a slow-growing scirrhous carcinoma of the breast, as contrasted with the rapid anæmia which follows the development of a small round cell sarcoma with its rapidly formed and abundant metastases.

The favoring of hæmorrhage is another condition by which the situation of the tumor may influence its action on the composition of the blood, as is best seen in tumors of the cervix and body of the uterus and of the gastro-intestinal canal, in which situations ulceration of the surface of the growth is very liable to occur, with erosion of the wall of a large vessel or abundant capillary oozing.

In general, it seems probable that the anæmia produced by a malignant growth may be referred to the toxic action on the blood and blood-making organs of the products of tne abnormal metabolism which goes on in the tissues of malignant tumors. Direct experimental proof of this by injecting extracts of tumors into animals has not as yet been obtained, but the immunity of animals to the results of transplantation of tumors derived from human beings may explain the lack of effect.

The great frequency of tumors of the stomach, and the marked anæmia which they may produce owing to their peculiar situation, which enables them to interfere with the digestion and the absorption of food, both by mechanically blocking the entrance and exit of the latter, and also by altering the digestion of the food by setting up a chronic gastritis, and their peculiar liability to hæmorrhage, warrant a special discussion of this form of anæmia.

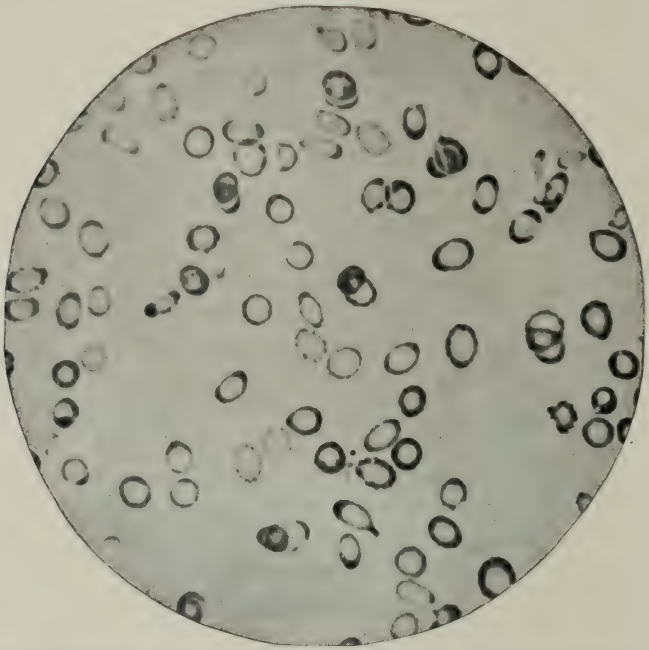

**Fig. 37.**—Secondary Anæmia due to Carcinoma of the Stomach. Magnified 700 diameters.

The blood in cases of carcinoma of the stomach shows no constant alteration which may be used for diagnostic purposes. The average change found is a considerable lowering of the hæmoglobin content of the red cells—in other words, a low color index—and of the specific gravity of the blood, without a very great reduction of the number of the erythrocytes or noticeable change in the shape and size of the latter—in other words, a secondary anæmia of a chlorotic type. The mild cases in which the tumor has not caused a marked stenosis of the pylorus or severe hæmorrhages, often show a red cell count of over 4,000,000, with a moderate leucocytosis in about half the cases and a few small or poikilocytic red cells. The severe cases may mimic pernicious anæmia very closely in their clinical aspect, though an examination of the blood will show over 1,500,000 red cells, while in pernicious anæmia of equal clinical severity the count would be much less. The cell type in carcinoma of the stomach is usually microcytic, the formation of fibrin is abundant, the blood clots quickly and the clot contracts well and squeezes out clear serum—phenomena often absent in pernicious anæmia. In most cases normoblasts can be found after a reasonable search, and occasionally a megaloblast when the anæmia becomes extreme. If metastases are present in the bone marrow the anæmia is likely to be very severe and show more megaloblasts than in a case without this condition.

Polychromatophilia and granular degeneration are present in the red cells in all well-marked cases. A moderate leucocytosis is present in about 30 per cent. of the early cases, and after the anæmia has become well established the proportion of the cases showing leucocytosis rises to about 60 per cent. The size and the situation of the tumor seem to influence the leucocytosis; small, slow-growing tumors often cause no leucocytosis, large, rapidly growing, pyloric tumors with metastases and cachexia favor leucocytosis. (Plate V, Fig. *B*, and Fig. 37, p. 144.)

A digestive leucocytosis is not present in about 90 per cent. of the well-marked cases, a point which has been suggested as of corroborative value in diagnosis. The relative proportions of the white cells are altered as the disease advances. The lymphocytes are relatively diminished, the polynuclears increased, averaging 70 to 75 per cent., the large mononuclears are slightly increased, or occasionally appear in very large numbers. Basophile cells may be increased up to about 3 per cent. This change is of great value as a differential diagnostic point between this form of secondary anæmia and pernicious, in which the lymphocytes show a relative or even an absolute increase.

A source of error which must be considered in the discussion of the results in the enumeration of the corpuscles and the determination of the hæmoglobin is the increase in the concentration of the blood which can often be observed after prolonged vomiting or in cases with marked dilatation of the stomach. This concentration results, it is evident, from the removal of large quantities of fluid from the body in the case of vomiting, or in the retention and non-absorption of such fluid as may be taken in, in the case of obstruction of the pylorus and great increase in the gastric capacity.

When a malignant tumor involves the bone marrow, either directly or by metastases, the blood picture which results is often quite characteristic. The anæmia is much more severe than when the marrow is not invaded. The number of nucleated red cells is likely to be greatly in excess of that usually seen in secondary anæmia due to malignant growths, and what is most characteristic of all, myelocytes of various types are usually seen in the blood.

A number of cases have been reported[1] in which a diagnosis has been made either of pernicious anæmia, as in the case of Ehrlich,[2] or in which myelogenous leukæmia was suggested, as in the case reported by Kast,[3] or in that of Strauss which suggested lymphatic leukæmia, a leucocytosis of 32,000 being found in a patient suffering from a general sarcomatosis, the white cells being almost entirely lymphocytes. As stated above, the variations in the red cells may be extreme, falling as low as 700,000 to the cubic millimeter. The leucocytes may or may not be increased in numbers. Usually there is a slight rise, sometimes as high as thirty or forty thousand. Normoblasts are almost always present; megaloblasts occasionally; poikilocytosis and polychromatophilia are more or less abundant. In two cases which the writer has had the opportunity to examine, the blood picture was not that of pernicious anæmia, and although occasionally megaloblasts could be found, the general morphology was distinctly that of a secondary anæmia. The neutrophile myelocytes in these cases were, in one, .5 per cent.; in the other 7 per cent. In some of the cases in the literature the neutrophile myelocytes have risen to 12 or 15 per cent. Eosinophile myelocytes, as a rule, however, are not over 1 to 2 per cent., and basophile cells are not greatly increased. Occasionally the neutrophile granules are very imperfectly developed even in the polynuclear forms. Besides the myelocytes, one occasionally meets with the peculiar basophilic mononuclears described by Türk as irritation forms. The hæmoglobin index in both of my own cases was low. The autopsy on one case showed myeloid changes in the spleen, and a few areas of myelocytes in the liver. The marrow was invaded by numerous small nodules of the carcinoma and the space between the tumor masses was filled by hyperplastic red marrow. In another case of gastric carcinoma, however, seen by the writer, though there were a few nodules of the tumor in the marrow of the long bones and a number of vertebræ had been invaded by direct extension, yet the changes in the blood were characteristic only of a moderate secondary anæmia and no myelocytes were found on making a differential count of the leucocytes. On the other hand, myelocytes in small numbers may be present even though the growth has not involved the marrow. Thus, in a case of carcinoma of the stomach, the writer found the red cells to be 3,628,000, the white 6,700, hæmoglobin 35 per cent. A differential count of a thousand cells was as follows: polynuclear neutrophiles, 70.9 per cent.; transitional, 5.6 per cent.; large lymphocytes, 13.5 per cent.; small lymphocytes, 8.5 per cent.; eosinophiles, 0.5 per cent.; basophiles, 0.7 per cent.; myelocytes, 0.3 per cent. It is therefore not always possible to diagnose the invasion of the bone marrow from the blood picture, as Kurpjuweit has assumed.[1]

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[1] *Kurpjuweit:* Deut. Archiv f. klin. Med., Bd. lxxvii, 1903, p. 553.

[2] Charité Annalen, vol. v, 1878, p. 198.

[3] Deut. Arch. f. klin. Med., Bd. lxxvi, 1903, p. 48.

### SECONDARY ANÆMIA DUE TO INTESTINAL PARASITES

**The Uncinaria duodenale** (Anchylostoma duodenale) is capable of producing severe and even fatal anæmias in those who harbor large numbers of worms in the intestinal tract. The changes in the blood in the mild infections are usually those of a simple anæmia of a chlorotic type with an increased number of eosinophiles. In more severe infections a marked anæmia may be present, the cells falling to two or even to one million to the cubic millimeter, with a moderate number of normoblasts and megaloblasts.

The color index is almost always low in contrast to pernicious anæmia and there is generally a moderate leucocytosis. The eosinophiles vary from normal numbers to as high as 72 per cent. of the leucocytes. The average in the cases studied by Boycott and Haldane[1] was 20.8 per cent.

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[1] Deut. Arch. f. klin. Med., Bd. lxxvii, 1903, p. 553.

The anæmia due to these worms is frequently met with in Northern Africa, where it is known as Egyptian chlorosis, and in Europe where it is termed tunnel-worker's disease. It is seen also in the Rhine Valley in the laborers in brickyards. In the United States the disease is occasionally met with in the coal miners in Pennsylvania[2] and in the Southern States. It is exceedingly frequent in Porto Rico and the Windward Islands.

**The Bothriocephalus latus** also causes severe anæmias. The blood change in these cases may be that of a mild chlorosis or secondary anæmia, or may be indistinguishable from pernicious anæmia with numerous megalocytes and megaloblasts. The poison of these worms has no positive chemotaxis on the eosinophile cells, at least after the production of a marked anæmia.[3]

The changes in the blood are, chiefly, a great reduction in the red cells, one of Schauman's cases having a count of 395,000. The average diameter of the cells is greater than normal. Poikilocytosis is well marked, as are also granular degeneration and polychromatophilia. Nucleated red cells are present in a large proportion of the cases and may be extraordinarily abundant, as in the case of Askanazy.[4] Megaloblasts are especially abundant, usually more so than normoblasts. The color index is high. Leucocytes are present in about normal numbers and there is no eosinophilia. Expulsion of the parasite usually results in a cure of the anæmia, but a number of cases have terminated fatally even though the parasite was removed from the intestine.

The production of the anæmia is probably due to the absorption of a hæmolytic substance derived from the bodies of the worms. Some observers have suggested that dead or diseased worms are those especially responsible for the production of anæmia, but this statement has not been verified. It is certain, however, that only a few persons carrying these worms suffer from severe types of anæmia, and it is probable that some congenital or acquired deficiency exists in the blood-making organs in those cases with fatal anæmia. The number of parasites present in the intestine is no index of the severity of the anæmia produced. An enormous number of parasites have been found in persons in good health.

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[1] Journal of Hygiene, vol. iii, 1903, p. 95.

[2] *Wainwright* and *Nichols:* Medical News, vol. lxxxiv, 1904, p. 785.

[3] *Schauman:* Bothriocephalus Anämie, Berlin, 1894.

[4] Zeit. f. klin. Med., Bd. xxvii, 1895, p. 492.

The diagnosis of the nature of both of these types of anæmia can easily be made by examining the fæces for the eggs of the respective worms, a procedure which should never be omitted in any case of severe anæmia.

### PERNICIOUS ANÆMIA[1]

The determining factors in the production of this disease are not known. A number of conditions have been frequently observed to precede the appearance of the characteristic changes in the blood, among which may be mentioned syphilis, malaria, chronic infectious diseases, severe hæmorrhages, either extending over some time or with a single large loss of blood, and, finally, intestinal parasites. Among the parasites, the Bothriocephalus latus and more rarely the Anchylostoma duodenale may stand in actual causal relation to the blood changes, for when they are removed by proper medication the blood condition may return to the normal. The same relation does not exist between the other diseases mentioned above and the anæmia, and the assumption that they act as determining agents is more than doubtful.

The changes in the blood are often exceedingly characteristic. The blood may be of normal color or exceedingly pale, at times it is very dark. The great relative increase in the serum as compared to the corpuscles makes the whole blood very thin and fluid. The preparation of suitable smears for the study of morphological changes is often a matter of considerable difficulty, but if the slide be warmed over a flame and the blood spread while the slide is still warm, crenation and other changes due to slow drying may be avoided. The specific gravity of the blood is greatly reduced in an advanced case and coagulation is retarded. The specific gravity of the serum is often but slightly changed.

The red cells are usually greatly reduced in number, at times

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[1] An excellent paper on this subject by *R. C. Cabot* will be found in Wood's Reference Handbook, Revised Edition. An exhaustive monograph containing a very complete bibliography will be found in *Ehrlich:* Die Anämie. Wien, 1898.

# DESCRIPTION OF PLATE VI

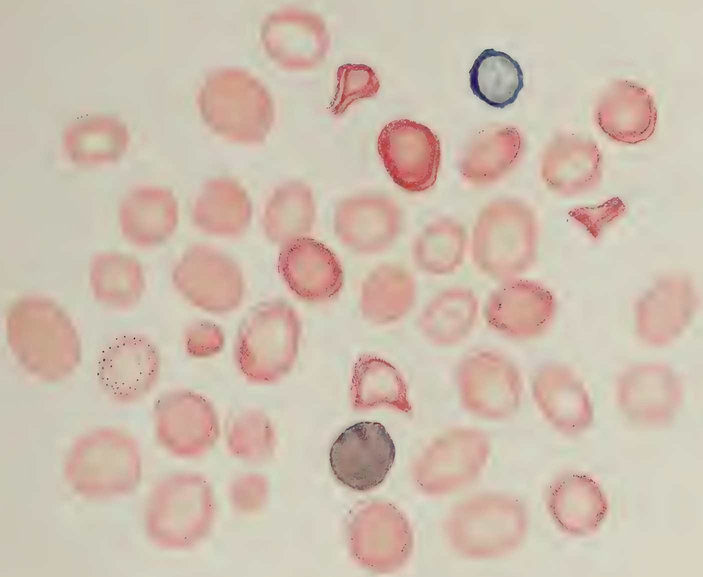
*Stained by the Jenner Method*

### *Figure A.*—Early Case of Pernicious Anæmia

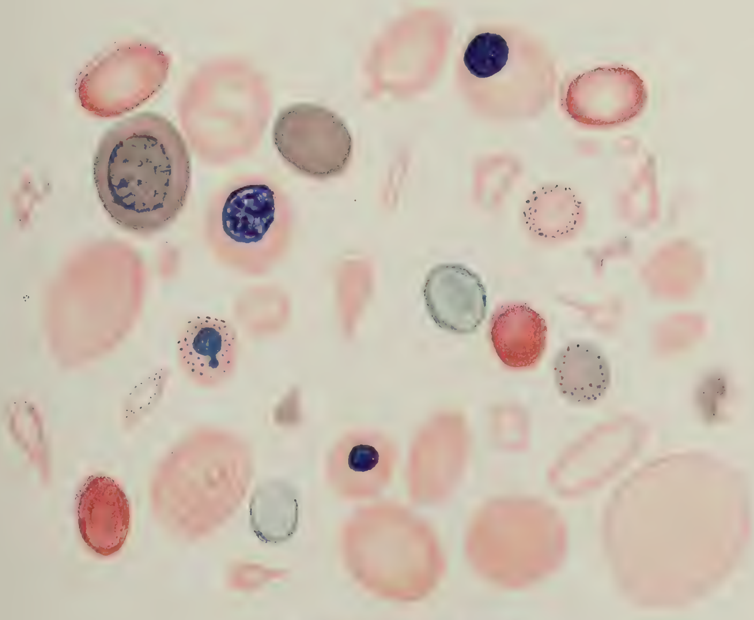
The characteristic change in the red cells is an increase in the size and in the amount of hæmoglobin contained in the individual corpuscle. This is well marked even in persons who are not clinically very ill. The patient from whom this blood was taken was a laborer who had not found it necessary to stop work, but merely felt unusually weak. Death did not occur until four years after this specimen of blood was obtained. Anisocytosis and poikilocytosis are always marked features of the blood in pernicious anæmia. Polychromatophilia is fairly abundant even in the early cases and occasionally basophilic granules may be seen in the red corpuscles. The leucocytes are not altered from the normal, or show a slight lymphocytosis.

### *Figure B.*—Advanced Case of Pernicious Anæmia

This blood is from a case of advanced pernicious anæmia and was taken only a few weeks before death. The picture is necessarily a combination one and does not show a single field of the microscope. A number of the photographs of mitotic figures in the text were obtained from the blood of this case. The relative size and proportions of the red cells are not in the least exaggerated. All the corpuscles drawn were measured carefully by a micrometer, and the drawing was made to a scale of 1,000 diameters. Poikilocytes and microcytes are abundant, some of the microcytes staining very deeply, others showing central depressions. The poikilocytes show similar variations in staining reactions and also polychromatophilia and punctate degeneration. In the lower left-hand corner is a large oval cell with a wrinkled surface. A photograph of a similar cell is shown in Fig. 30. In the polychromatophilic megaloblast shown in the upper left-hand corner, the nucleus has a well-marked chromatin network and corresponds to Engel's metrocyte of the first generation. A megaloblast corresponding to a metrocyte of the second generation is shown in the upper right-hand corner of the figure. Several normoblasts with pycnotic nuclei may also be seen in the lower portion of the figure. Megalocytes of extreme size such as may be seen in the lower right corner of the plate are very rare and only seen as a terminal phenomenon.



A.—EARLY CASE.



B.—ADVANCED CASE.

PLATE VI.—PERNICIOUS ANÆMIA.

to an extraordinary degree, the minimum on record being 143,000 per c.mm. Numbers between five hundred thousand and eight hundred thousand are not uncommon, but the general average for a well-marked case is about one million. Cases having well-marked clinical symptoms and advanced morphological changes in the blood may show three million red cells, and others in which the count is much lower, even as low as five hundred thousand, may not give evidence of much distress and be up and about.

The examination of the fresh blood shows an almost complete absence of rouleau formation and the presence of many poikilocytes. The blood plates are also scarce and the fibrin scanty in amount. The examination of stained smears is required, however, to show properly the characteristic changes in the red cells. The first point to be noted is that the disks of many of the red cells have a very slight central depression, or often no depression at all, while the cell stains a very deep red with eosin or the acid fuchsin of the Ehrlich triacid stain, though occasionally, especially in the poikilocytic forms, the central depression is very marked. If it

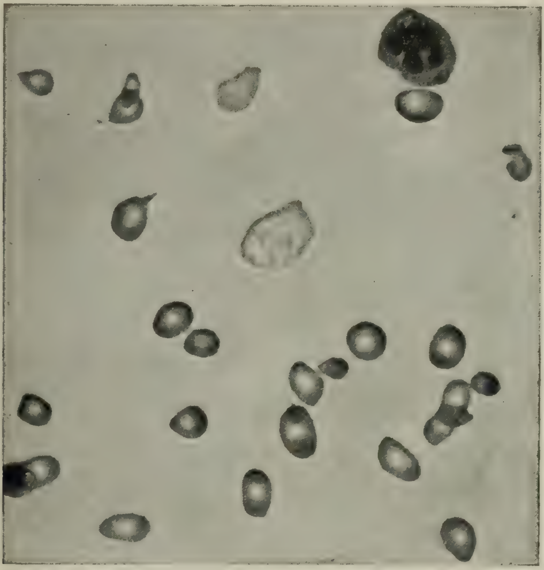


Fig. 38.—Pernicious Anæmia. Poikilocytosis and Anisocytosis. Vacuolar Degeneration of Large Cell in Center. Magnified 700 diameters.

be remembered that the hæmoglobin of the red cell is the component which combines with these dyes, it will be evident that the hæmoglobin of the deeply staining cells is greatly increased, in direct contrast to the faint ring-like cells seen in chlorosis. This increase is also noted in the determination of the total hæmoglobin as made by the hæmoglobinometer and compared with the number of cells obtained by enumeration. The red cells will be found, as a rule, to be diminished to a greater extent than the hæmoglobin, or, in other words, the hæmoglobin index is high.

The size of the cells is next to be noted, and if careful determinations be made of the diameters of a series of cells selected indiscriminately, it will be found that the average diameter of the cells is increased in distinction to the condition found in chlorosis and the secondary anæmias, where the average diameter is decreased. (See Plates II and VI, also Figs. 30, 33, and 38.)

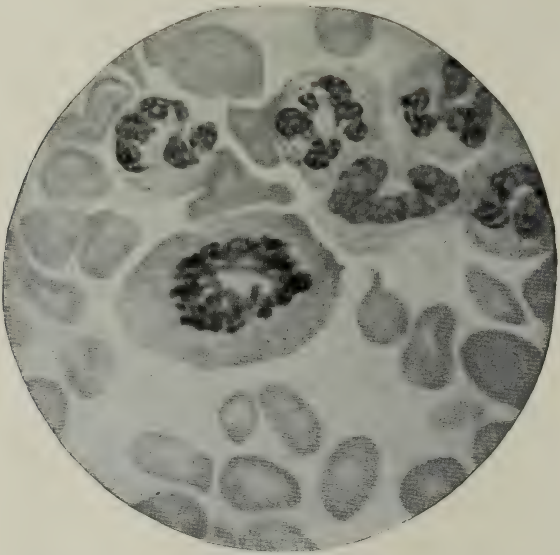


Fig. 39.—Mitosis in a Megaloblast. Pernicious Anæmia. Monaster Stage. Magnified 1,000 diameters.

Macrocytes or megalocytes are abundant even in early cases, and vary a good deal in their morphology. They may be simply cells of normal form measuring over 11 micra in diameter, or they may be very faint in their staining qualities, or they may even stain deeply and contain an excess of hæmoglobin. In advanced cases very large megalocytes may be seen which show a distinct wrinkling of the cell in the smear, or large, irregular vacuoles in the substance of the protoplasm (Fig. 38). Often a faint network can be seen in the center of the megalocytes, showing where the nucleus has been, though so far absorbed and altered by the process of karyolysis[1] that the nuclear substance does not react to the nuclear stain (Plate II, Figs. 28 and 30). Microcytes vary a good deal; they are usually present in moderate numbers. Poikilocytes are very abundant in all cases and involve all types of cells, even the nucleated forms. There is a general tendency for the cells to assume a slightly oval shape, even if they are not irregular enough to be classed as poikilocytes. The nucleated cells are of three types, of which the megaloblasts are the most abundant, the normoblasts less so, and the microblasts rather infrequent. For convenience of classification it is well to assume as megaloblasts all nucleated red cells over 11 micra in diameter.

If a careful enumeration of the nucleated cells be carried out, it will be found that in pernicious anæmia the megaloblasts, as a rule, are more abundant than the normoblasts, the exact reverse of the condition in severe secondary anæmias, where megaloblasts are very rare and normoblasts the common type of the nucleated cell. The normoblasts show no especial morphological differences from those of the bone marrow and of the secondary anæmias. The nuclei are often pycnotic, round or lobular, or arranged in a clover-leaf form, or divided into two or more nuclei (Plate II, Figs. 16 to 23, Plate VI, Fig. *B*).

Polychromatophilia and granular degeneration are very common, and some of the normoblasts in which the polychromatophilia is well advanced can only be distinguished with great difficulty from the small lymphocytes (Plates II and III, Figs. 63 and 81).

The megaloblasts[2] usually possess the characteristic net-like arrangement of the nuclear chromatin, through the interstices of which may be seen the stained hæmoglobin of the cell body (Plate II, Figs. 23 to 32). The nucleus is large in most examples, occupying the greater portion of the cell which surrounds it as a narrow border and which frequently shows the most marked degree of polychromatophilia. Occasionally, however, the nucleus may be small and pycnotic though the cell is large (Plate II, Figs. 27, 29, 31). The outline of the cell body may be sharp, but usually it is poorly defined and fringe-like (Fig. 26). In rare cases mitotic figures may be seen in the megaloblasts, and occasionally in the normoblasts[1] (Plate II, Figs. 33 to 37). There is often a well-marked retraction zone about the edge of the nucleus in stained specimens where the nucleus has become separated from the cell body by shrinkage of the preparation.

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[1] A term applied to the process by which a nucleus is dissolved without being broken in fragments.

[2] A general discussion of the recent views concerning the pathological importance of these cells may be found in *Bloch:* Ziegler's Beiträge, Bd. xxxiv, 1903, p. 331.

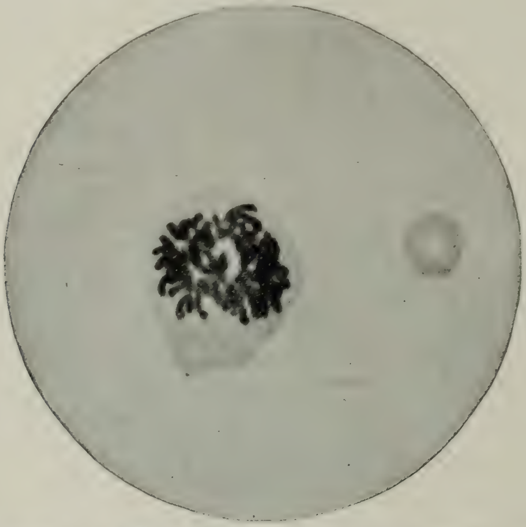

**Fig. 40.—Mitosis in Megaloblast in Pernicious Anæmia. Monaster Phase.** Magnified 1,000 diameters.

The microblasts require no especial description (Plate II, Fig. 21). The cell body is often very narrow and strongly polychromatophilic, and at times may entirely disappear, leaving the nucleus free in the serum.

The white cells in pernicious anæmia are diminished often to a considerable extent, the lowest recorded figure being that reported by Lazarus[2] as three hundred and thirty to the cubic millimeter, though there is some doubt as to the exact figures, the proportion between the red and white cells being given without an actual record of count. The average of the writer's cases is about four thousand.

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[1] *Askanazy:* Zeit. f. klin. Med., 1893, Bd. xxiii, p. 80.

[2] *Ehrlich* u. *Lazarus:* Die Anämie. Wien, 1898.

The relative proportions between the leucocytes are altered, the lymphocytes being considerably increased and the polynuclear neutrophiles diminished. That this does not always hold to a marked degree is shown by a case of the writer's in which the blood was as follows: Red cells, 1,080,000; white cells, 7,000; hæmoglobin, 20 per cent.; polynuclear neutrophiles, 65 per cent.; large lymphocytes, 11 per cent.; small lymphocytes, 22 per cent.; eosinophiles, 1.3 per cent.; basophiles, 0.6 per cent. About an equal number of normoblasts and megaloblasts were present.

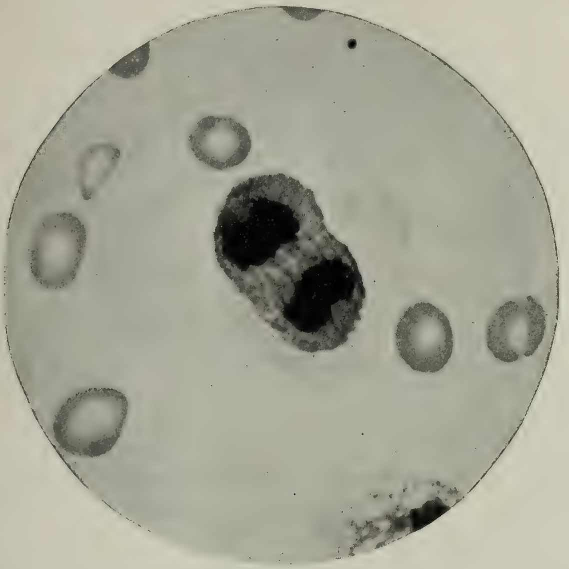

FIG. 41.—MITOSIS IN MEGALOBLAST IN PERNICIOUS ANÆMIA, DIASTER STAGE. NOTE THE GRANULAR APPEARANCE OF THE CYTOPLASM. Magnified 1,000 diameters.

Rarely a considerable increase in the number of leucocytes is seen which is usually temporary and often associated with a "crisis" of normoblasts or megaloblasts or may accompany an intercurrent infection (Fig. 33). Occasionally it is an agonal process. Myelocytes are often seen in small numbers. Conclusions should not be drawn from a stained smear as to the number of leucocytes, since they may appear as increased, owing to the great relative decrease in the number of the reds.

The hæmoglobin is not reduced in proportion to the reduction in number of the red cells; that is, if the red cells are reduced to 20 per cent. of the normal, the hæmoglobin will probably be about 20 per cent. or a little above it; exceptions, however, occur. The reason for this condition, which is quite characteristic of pernicious anæmia as compared to the secondary anæmias, is that the numerous megalocytes present contain relatively more hæmoglobin than the corresponding number of normal sized cells, and thus raise the hæmoglobin per cent.

The iron content of the whole blood is greater than the amount of hæmoglobin would lead one to expect, showing that both the serum and the red cells contain iron in some form independent of the iron contained in the hæmoglobin of the red cells. This is probably due to the very great destruction of the red cells which takes place in pernicious anæmia, as evidenced by the marked

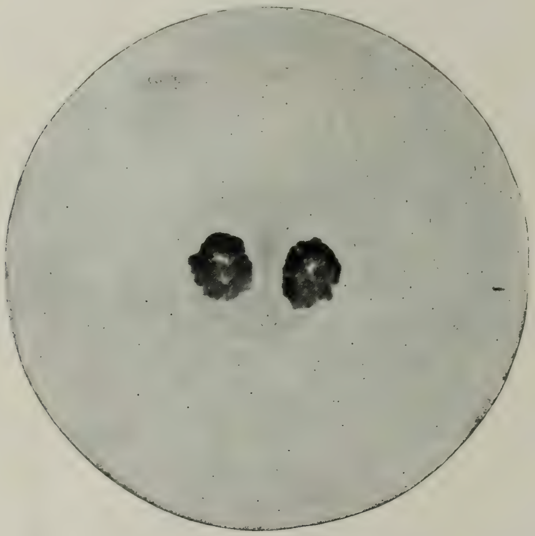

Fig. 42.—Mitosis in Red Cell. Nuclear Division is Completed. Segmentation of the Cell Body not yet Begun. Magnified 1,000 diameters.

increase of iron in the urine and the constant presence of large quantities of urobilin in that fluid.

The **diagnosis** of a severe pernicious anæmia may often be made from the examination of a stained smear even though the clinical picture is not characteristic; the diagnosis of a mild case is often a matter of some difficulty and requires a careful count of both red and white cells and a hæmoglobin determination. The chief points in the morphology of the blood upon which a diagnosis may be based, are the general prevalence of deeply staining megalocytes and of megaloblasts, even though the number of the latter be few. A case is occasionally met with in which no megaloblasts can be found even after a prolonged search, though the

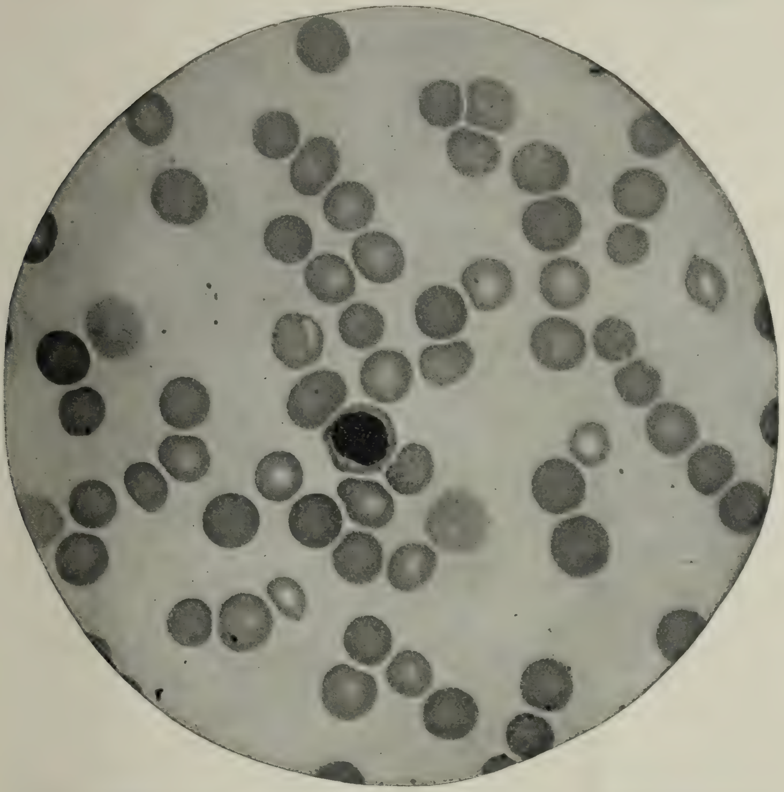

Fig. 43.—Pernicious Anæmia in Stage of Remission. Same Case as Fig. 33. A Lymphocyte is in center of Field. Magnified 800 diameters.

marrow shows large numbers of these cells. The low leucocyte count and relative lymphocytosis so often present in this form of anæmia are also valuable in distinguishing mild cases from severe secondary anæmias.

If megaloblasts can not be found after a careful search, the examination should be repeated in a few days, as marked remissions often occur in this disease, during which the blood may lose many of its abnormalities, and especially its content of large and nucleated red cells. This same phenomenon is often seen a few days before the death of the patient, the megaloblasts disappearing from the circulation, though present in the bone marrow, while a moderate leucocytosis may appear.

Care should also be taken by repeated and careful study of the blood to avoid mistaking a case of either lymphatic or myelogenous leukæmia in the stage of remission for a pernicious anæmia. The leucocytes in leukæmia may be reduced to normal limits by treatment or an acute infection, and unless a careful differential count is made an error is quite possible. The differential will show a large percentage of myelocytes in the myelogenous form and a large number of lymphocytes in the lymphatic, while in pernicious anæmia myelocytes are few and lymphocytes are rarely above 60 per cent.

The differential points between pernicious anæmia and a severe secondary anæmia, which may be clinically quite indistinguishable, are the leucocytosis often present in the secondary and the low hæmoglobin index of the red cells so constantly found in secondary anæmia and so rarely found in pernicious. In the secondary anæmias also the average red cell is small, rather than large, as is the case in pernicious anæmia. There is no method of distinguishing between the severe types of anæmia incited by the parasitic worm Bothriocephalus latus and the most typical pernicious anæmia, except by the examination of the patient's stools, as the eosinophiles are not increased in the severe worm anæmias, which resemble pernicious anæmia in the morphology of the blood, and a leucocytosis is also not constantly present.

### ANÆMIA IN CHILDREN

The diagnosis of the form of an anæmia in young children is often rendered difficult, if not impossible, by a number of circumstances. First among these is the great normal variability of the child's blood. The red cells often fluctuate between 4,000,000 and 6,000,000 and the hæmoglobin varies from day to day even as much as 15 per cent. The leucocytes are easily increased in numbers by very slight stimuli.

A megalocytic type of blood is frequently seen, which only means a severe anæmia and does not indicate a pernicious anæmia, as would such a phenomenon in an adult. The appearance of nucleated cells has also far less significance than in the blood of adults; even megaloblasts may be seen without a necessarily grave prognosis to the case. The writer, for example, has seen numerous normoblasts appear in the blood of a three-year-old child during the course of a pneumonia, while a similar phenomenon has been noted in severe sepsis.[1]

Myelocytes with eosinophile or neutrophile granulations are occasionally present and do not indicate a leukæmia, only a severe anæmia or a grave infection. Enlargement of the spleen is common in anæmias due to a variety of conditions, such as syphilis, rickets, tuberculosis, etc.

Pernicious anæmia is a rare disease in children, and the blood changes are much more marked than in similar conditions in adults. The number of nucleated red cells of all types may be enormous and mitotic figures in the nuclei may be very abundant.[2] Poikilocytosis is common in conditions in which no such change would be seen in an adult. Many cases described as pernicious are no doubt instances of severe secondary anæmia of unknown cause.[3]

Leukæmia in infancy is rare, not more than ten well-observed cases existing in the literature. The disease can be easily diagnosed by the high leucocytosis and the differential count. The usual type is lymphatic, often acute and of a hæmorrhagic form, but a few myelogenous cases are on record.

### INFANTILE PSEUDOLEUKÆMIA

Under this title von Jaksch[4] described a rare form of disease seen in infants,[5] characterized by a very marked anæmia, enlargement of the spleen and occasionally of the liver and lymph nodes, with an increase in the leucocytes of from 20,000 to 50,000, or more rarely to 100,000, to the cubic millimeter. The morphological changes in the blood resemble those seen both in pernicious anæmia and in leukæmia. Large numbers of nucleated red cells are present, both of normoblastic and megaloblastic types, and mitotic figures are often abundant. The leucocytes are chiefly mononuclear in form and myelocytes are present in moderate numbers. The disease may terminate fatally, or pass into an anæmia of the pernicious type, or into leukæmia, or the child may recover. In fatal cases myeloid changes have been noted in the liver, while a chronic hyperplasia of the spleen has been met with though not constantly. One of von Jaksch's cases showed leukæmic changes in the viscera. The lesions of syphilis and rachitis are frequently found, while many of the infants in the observed cases have suffered from gastro-intestinal disturbances.

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[1] *Raybaud* et *Vernet:* C. R. Soc. d. Biol, 1903, p. 672.

[2] *Theodor:* Arch. f. Kinderheilkunde, 1900, p. 321.

[3] See for a case of this type, *Rotch* and *Ladd*, Arch. of Pediatrics, 1901, p. 641.

[4] Wien klin. Woch., 1889, p. 435 and 456, and Prag. med. Woch., 1890, p. 389.

[5] One of the best reviews of this subject with the literature is by *Wentworth*, in the Boston Med. and Surg. Journal, vol. cxlv, 1901, pp. 372, 402, 435, 461, 488. *Morse:* Boston Med. and Surg. Jour., vol. cxlviii, 1903, p. 573.

The current opinion at present seems to be that this disease is a secondary anæmia corresponding to the anæmia splenica infettiva of the Italians, and that the alterations in the morphology of the blood do not indicate so advanced a lesion as do the corresponding changes in adults. A differential diagnosis from pernicious anæmia is difficult, as in children megaloblasts and a leucocytosis are seen in both conditions. In leukæmia of a myelogenous form in children the liver is enlarged, while in the lymphatic type the hyperplasia of the lymph nodes is marked in distinction to pseudoleukæmia, where the nodes are usually not enlarged.

## PSEUDOLEUKÆMIA (HODGKIN'S DISEASE)

This disease is usually described with the leukæmias because of the close resemblance of its clinical picture to that of lymphatic leukæmia. Its diagnosis depends much more upon the clinical features of the disease, and the presence of discrete, freely movable, enlarged lymph nodes than upon the blood examination. The usual type shows a general hyperplasia of the lymph nodes of the body accompanied by a moderate anæmia of the chlorotic type, that is, with a relatively high number of red cells and a low hæmoglobin per cent. Poikilocytosis and degenerative changes in the red cells are not marked. The leucocytes are generally assumed to show no quantitative or qualitative changes, but recently it has been claimed[1] that all cases of pseudoleukæmia have a distinct relative lymphocytosis which enables this disease to be easily distinguished from other conditions resembling Hodgkin's disease, especially from those cases in which tubercle bacilli have been found in the lymph nodes of patients otherwise running a course perfectly typical of true Hodgkin's disease. My own observations on seven cases and those of DaCosta,[2] Longcope,[3] and others have not confirmed this statement, It is certain that a few cases do show a relative lymphocytosis, but these are in the minority. The nodes in pseudoleukæmia usually remain discrete and show a marked hyperplasia either of the lymphoid or of the large endothelial cells, in distinction to the nodes in cases of lymphosarcoma, which soon grow through their capsules and involve the surrounding tissues as a true malignant growth. In lymphosarcoma there is usually a considerable increase in the lymphocytes in the circulation, though a great reduction may be observed even to as low as .5 per cent., where all the lymphatic apparatus is involved by the tumor, thus excluding from use the tissues which form the lymphocytes. Shortly ante mortem, in Hodgkin's disease, there may be a great increase in the number of leucocytes without any morphological evidences of a transition to a true lymphatic leukæmia, though in a very few instances such a phenomenon has been observed.

### SPLENIC ANÆMIA

Under this designation are collected an obscure group of anæmias which are characterized by a relatively high red cell count, a marked reduction in the hæmoglobin percentage, and no constant increase in the leucocytes, which may, however, show a relative lymphocytosis and occasionally an increase in basophiles. Poikilocytes and nucleated red cells, both of normoblastic and megaloblastic types, are very uncommon, though one case of Osler's showed six hundred blasts to the cubic mm. The striking clinical symptoms are the enlargement of the spleen which precedes the anæmia, and the severe gastric hæmorrhages which seem to be frequent in this condition. The spleen is usually of large size, reaching to the umbilicus or below, while the lymph nodes are not increased in size. A differential diagnosis from hepatic cirrhosis is often extremely difficult, since the liver may be enlarged though not cirrhotic, and depends more upon the general appearance of the case than upon any definite symptom or change in the blood morphology. Osler lays stress on the facies in alcoholic cirrhosis, the more moderate enlargement of the spleen, and the course of the disease.[1] In two fatal cases seen by the writer, one of which had been considered as splenic anæmia, the liver showed an interstitial hepatitis which could not be distinguished from alcoholic cirrhosis, and also a chronic splenic hyperplasia with the formation of a good deal of new connective tissue.[2] Such cases correspond to the type of anæmia described by Banti [3] which begins with an enlargement of the spleen, followed by anæmia, then later by ascites and the symptoms of an atrophic cirrhosis. The fatal cases have shown a chronic splenitis, marked interstitial hepatitis, and occasionally red bone marrow. The anæmia is of the chlorotic type with no characteristic changes in the leucocytes.[4]

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[1] *Pinkus:* Pseudoleukämie. Wien, 1901, p. 84. (Nothnagel's Spec. Path. und Therap. Bd. viii.)

[2] Clinical Hæmatology, 1902, p. 268.

[3] Bull. Ayer Clin. Laboratory. No. 1, 1903. An excellent paper on the subject of Hodgkin's disease. See also *Simmons*, Journal of Med. Research, 1903, N. S., vol. iv, p. 378.

## LEUKÆMIA

The inciting factors in this disease are still quite unknown, and although the general appearance of the acute lymphatic leukæmias is that of an infection, yet the bacteria which have been occasionally isolated from the blood seem to have no causal connection with the condition. Löwit [5] has found bodies in the cells which he considers to be protozoa, but this observation has not been verified by others. The blood changes are at present assumed to be a continuous and extreme leucocytosis which affects, in one type of the disease, the lymphocytes, and in the other type the granular cells, causing, by some chemotactic action as yet not understood, the entrance of these cells from the blood-forming organs into the blood. In general two types of the disease are distinguished, based upon the appearance of the blood of these two types of cells—in other words, lymphatic and myelogenous leukæmia. The lymphatic may be acute or chronic; the myelogenous is practically always chronic, though the course of the disease may be short, and a few observers have recorded cases which they consider as of the acute myelogenous variety.

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[1] Amer. Journ. of Med. Sciences, vol. cix, 1900, p. 54; ibid., vol. cxxiv, 1902, p. 751.

[2] *Sippy:* ibid., vol. cxviii, pp. 428 and 570, gives an excellent review of the literature of pseudoleukæmia.

[3] Beit. v. Ziegler, Bd. xxiv, 1898, p. 21.

[4] *Borissowa:* Virch. Arch. Bd. clxxii, 1903, p. 108.

[5] Die Leukämie als Protozoeninfection, 1900.

For a recent review of the theories of the pathology and classification of the different types of leukæmia, see *G. Banti:* Cent. f. Path., 1904, p. 1. See also *Pappenheim*, Zeit. f. klin. Med., Bd. lii, 1904, p. 257.

The blood as a whole in leukæmia shows certain changes common to all types. When seen in the fresh condition, the large number of leucocytes often gives to the drop a very pale color, even a milky appearance. When it coagulates in the larger vessels or in the air, it separates, in cases rich in leucocytes, into two layers, one containing the white cells mainly, the other the red. The coagulation in some cases, though not constantly, is considerably delayed and so imperfect that a true clot is not formed. The specific gravity of the blood may be considerably reduced owing to the anæmia which is a constant factor in the disease. It may fall as low as 1.030 or even 1.023. The alterations in the cells are best described under the different types of the disease. The hæmoglobin is always low. In the myelogenous and chronic lymphatic forms the hæmoglobin index is also reduced, while in the acute forms a high index is occasionally met with.

## ACUTE LYMPHATIC LEUKÆMIA

Clinically this disease resembles most closely an acute infection, often simulating typhoid fever, a hæmorrhagic sepsis, or scurvy. There is a rapidly increasing anæmia, a moderate elevation of temperature, hæmorrhages into the skin, mucous membranes, or the viscera, with enlargement of the lymph nodes and the lymphoid tissue in the intestines, and a moderate increase in the size of the liver and spleen. A few cases are on record in which there was no increase in size either of the lymph nodes or of the other organs.[1] Leukæmic nodules in the skin are not infrequent, which show microscopically only an infiltration of the subepithelial tissues with lymphocytes. Some of the cases have given a septic temperature; others show a curve characteristic of typhoid; and a diagnosis of malignant endocarditis has been made.[1] The blood changes are very characteristic. The red cells rapidly diminish in number, often to below two million; normoblasts are seen; other forms are rare. In one case which the writer has examined the changes in the red cells resembled slightly those seen in pernicious anæmia, and megaloblasts were not infrequent.

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[1] *Kelly:* Univ. of Penn. Medical Bull., 1903, p. 27.

The leucocytosis is moderate, often below 100,000, though high counts are recorded. The cells present are chiefly lymphocytes, either large or small; myelocytes in small numbers may or may not be present; eosinophiles are relatively diminished; polynuclears and large mononuclears are present in variable numbers. The hæmoglobin is reduced about in proportion to the reduction of the red cells. The striking point in the morphology of the blood is the presence of the large forms of lymphocytes, which are about the size of the large mononuclears seen in normal blood, measuring up to 15 micra in diameter. The cell body in these lymphocytes is narrow, of basophilic reaction, and shows the lobular projections characteristic of the larger lymphocytes in normal blood; the nucleus may be oval, or it may be notched or even divided into two lobes. In the large forms a marked degeneration is often seen, both the nucleus and the cell body staining very feebly, the cell body at times not at all—a peculiarity which may explain the statement of some French observers[2] that there is a form of leukæmia in which the cells are of the large mononuclear type, as these degenerated lymphocytes resemble somewhat the large mononuclear forms.

An acute intercurrent infection will usually reduce greatly the number of white cells in the blood and there may be a relative increase in the polynuclear neutrophiles. If the process is of long standing the characteristic lesions in the bone marrow may disappear and show no evidence of leukæmia post mortem.[3] The leukæmic skin nodules may also disappear under these conditions.

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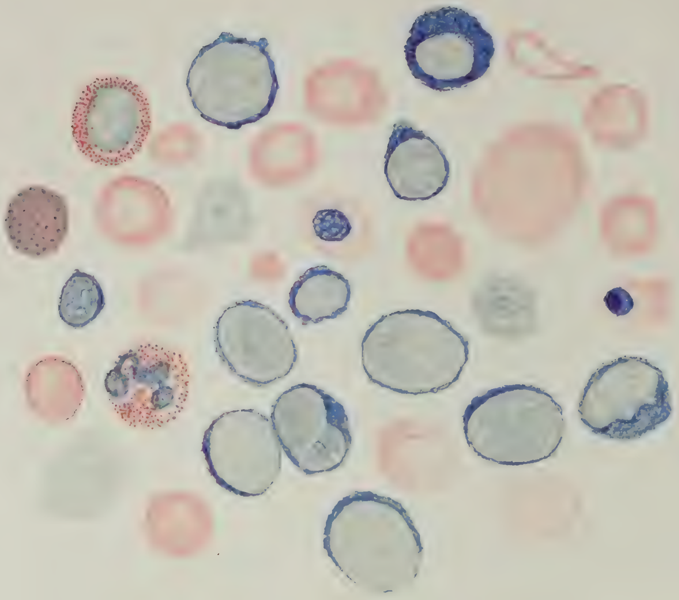
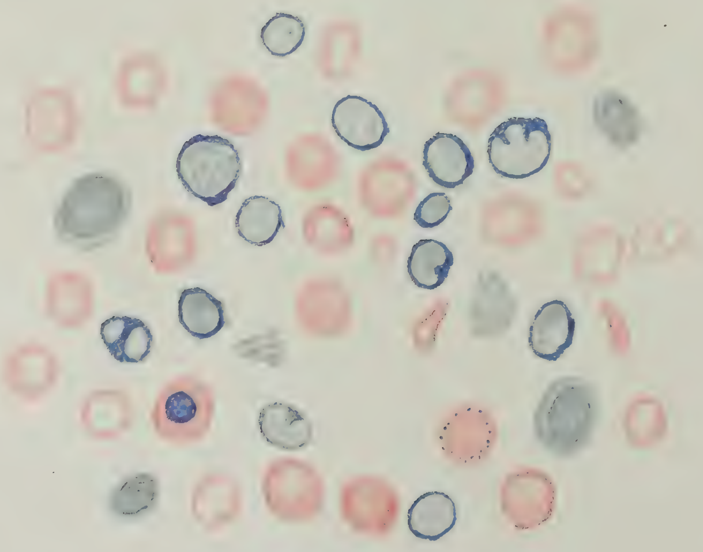
[1] *Bloch:* Deut. med. Woch., 1903, p. 512.

[2] *Lion:* Semaine Médicale, 1900, p. 91.

[3] *Körmöczi:* Deut. med. Woch., 1899, p. 773.



1.—ACUTE LYMPHATIC LEUKÆMIA.



2.—CHRONIC LYMPHATIC LEUKÆMIA.

PLATE VII.—LYMPHATIC LEUKÆMIA.

# DESCRIPTION OF PLATE VII

## *Stained by the Jenner Method*

### *Figure A.*—Acute Lymphatic Leukæmia

The red cells in acute lymphatic leukæmia often show very marked changes such as are usually considered characteristic of pernicious anæmia. There is abundant poikilocytosis and anisocytosis together with numerous nucleated red cells of a megaloblastic type. The characteristic phenomenon is, however, the presence of numerous lymphocytes chiefly of the large variety. These cells may measure 10 to 15 micra or even more in diameter. The nucleus usually takes a faint stain and the cell body a deeper one. Nucleoli are frequently demonstrable by the Jenner stain. The nucleus may be incurved or two-lobed or broken up into numerous fragments, but this change is not frequently seen. A few polynuclear neutrophiles and occasionally an eosinophile may be seen, and in some cases a considerable proportion of myelocytes, chiefly of the neutrophile variety. Degenerated leucocytes are abundant. An example is figured in the lower left-hand corner of the plate.

### *Figure B.*—Chronic Lymphatic Leukæmia

In this condition the red cells show a moderate anæmia and a few normoblasts may be present, but the changes are not so marked as in the acute disease. The lymphocytes are chiefly of the small variety and may be normal or show fragmentation of the nucleus and irregular staining of the cell body. Degenerated forms are frequently met with.

## CHRONIC LYMPHATIC LEUKÆMIA

Chronic lymphatic leukæmia is a disease running a course extending over a number of months or years, and more often accompanied by considerable enlargement of the spleen and the lymph nodes than is the acute form. The morphology of the blood also differs somewhat from that of the acute cases. The red cells are, as a rule, not as greatly diminished as in the acute form; the number of nucleated red cells is not usually so great, and they are chiefly normoblasts. Crises of normoblasts have been noted, as is well shown in the photograph from one of the writer's cases. The normoblasts reached the unusual number of over forty thousand to the cubic millimeter. The hæmoglobin is diminished more than the red cells. The white cells average about one

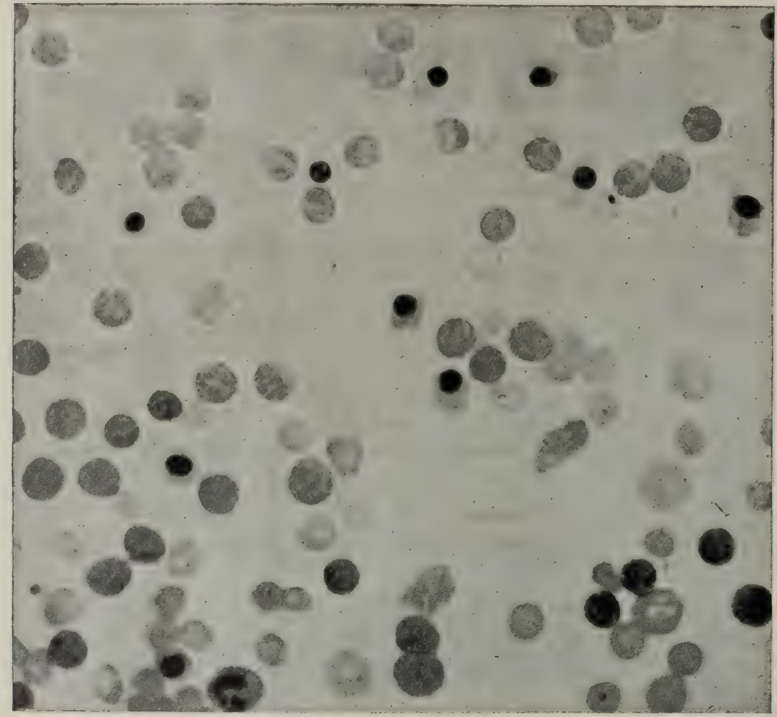


Fig. 44.—Normoblastic Crisis in Lymphatic Leukæmia. The Cells with Small, Dark Nuclei are Normoblasts. Magnified 700 diameters.

hundred thousand to the cubic millimeter, though counts of 400,000 or 500,000 are not uncommon according to the writer's experience. The white cells are chiefly lymphocytes of the small type found in the normal blood, and make up from 90 to 98 per cent. of all the leucocytes. They stain, as a rule, fairly well, though occasionally pale, degenerate, or atypical forms, with irregular, indented, or divided nuclei, are seen. Eosinophile and neutrophile cells and myelocytes are seen, but form but a small part of the total.

The leucocytes are subject to great variations, which may appear in counts made from day to day. With intercurrent infections, such as pneumonia, the number of the white cells may be reduced to the normal, only to rise again after the disappearance of the fever. Müller, however, records a marked increase in a patient with lymphatic leukæmia.

Concerning the site of the formation of the lymphocytes there is much discussion, two possibilities being debated. One is that these are formed in the lymph nodes and lymph tissue scattered about the body; the other is that they are formed in the bone marrow, as are all the other leucocytes of the blood. The reason for doubting the generally received theory of the lymph node origin of the lymphocytes is that cases of undoubted lymphatic leukæmia have been described in which there was no enlargement of the lymph nodes, and also that in Hodgkin's disease, in which there is a great hyperplasia of the nodes, there is no increase in the number of lymphocytes in the circulation. On the other hand, it has recently been shown that in both acute and chronic lymphatic leukæmia there is a very constant alteration of the fatty marrow of the long bones into a tissue resembling lymph tissue.

### ACUTE MYELOGENOUS LEUKÆMIA

The existence of an acute form of myelogenous leukæmia has been considered as unproven until recently when a sufficient number of observations have been collected to enable us to note the more important features of the disease. Owing to the small number of cases reported and the variability of the blood findings, the following table, modified from one by Billings and Capps, has been inserted. It is interesting to note that in a considerable proportion of the cases the disease was accompanied by ulceration of the

| No. | Observer | Sex Age | Duration | Mouth Infection | Lymph Nodes |
|---|---|---|---|---|---|
| 1 | Van der Wey, 1896. | F. 23 | 3 months. | None. | Cervical, submaxillary, and inguinal, vary in size from a pea to a bean. |
| 2 | Thompson and Ewing, 1898. | F. 21 | 8 weeks. | Sore throat. | Normal. |
| 3 | Grawitz, 1900. | F. 42 | 2 weeks. | Stomatitis; necrosis of jaw. | Moderate enlargement of cervical group. |
| 4 | Ewing, 1901. | M. 34 | 2 months. | Gingival; hæmorrhage from mouth. | Slightly enlarged. |
| 5 | Ewing, 1901. | — | Acute course. | Not stated. | Slightly enlarged. |
| 6 | Hirschfeld and Alexander, 1902. | M. 21 | 2 months. | Necrosis of hard palate and lip. | Cervical, size of pea to pigeon's egg. |
| 7 | Michaelis, 1902. | F. 50 | 15 weeks. | None. | Normal. |
| 8 | Billings and Capps, 1903. | M. 68 | 2 months. | Caries of jaw. | Slight enlargement of the cervical. |
| 9 | Türk, 1903. | — | —— | —— | —— |

(1) *Van der Wey:* Deutsch. Arch. f. klin. Med. 1896, p. 287.
(2) *Thompson* and *Ewing:* New York Medical Record, 1898, vol. liii, p. 333.
(3) *Grawitz:* Pathologie des Blutes, 1902, p. 318.
(4) *Ewing:* Clinical Pathology of the Blood, 1903.
(5) *Ewing:* ibid.

| Spleen | White Count | Differential Count of Leucocytes |
|---|---|---|
| Palpable. | 175,000 to 300,000 | Few small mononuclears; polynuclears, 33 per cent.; eosinophiles, 1.9 per cent.; myelocytes, 60 per cent.; containing faint and distinct granules.<br>Polynuclears, 3.7 per cent.; eosinophiles, 0.3 per cent.; mononuclears and myelocytes, 96 per cent. The large mononuclears predominate, although myelocytes are plentiful. |
| Scarcely palpable. | 50,000 to 100,000 | At first, myelocytes, 5 per cent.; five days later, lymphocytes, 33 per cent.; polynuclears, 55 per cent.; myelocytes, 12 per cent.<br>After death: Lymphocytes, 17 per cent.; polynuclears, 48 per cent.; eosinophiles, 0.3 per cent.; myelocytes, 17 per cent.; many with indistinct granules. |
| Three fingers below costal border. | 190,000 | Small mononuclears, 5 per cent.; large mononuclears, 15 per cent.; polynuclears, 10 per cent.; myelocytes, 60 per cent. |
| Moderately enlarged. | Marked increase. | Neutrophile myelocytes in great numbers. In other respects the blood was typical of myelogenic leukæmia. |
| Moderately enlarged. | 31,000 | Small mononuclears, 61 per cent.; polynuclears, 6 per cent.; neutrophile myelocytes, 31 per cent.; eosinophile myelocytes, 2 per cent. Few mast-cells. |
| One finger below costal border. | 42,000 | Small mononuclears, 7.4 per cent.; large mononuclears, 19.2 per cent.; polynuclears, 63 per cent.; eosinophiles, 0; myelocytes, 8.6 per cent. |
| Very large. | 16,000 | Small mononuclears, 20 per cent.; large mononuclears, 56 per cent.; polynuclears, 16 per cent.; eosinophiles, 0.4 per cent.; mast-cells, 0.4 per cent.; neutrophile myelocytes, 7.2 per cent. |
| One finger below costal border. | 540,000 to 374,000 | Small mononuclears, 4 per cent.; large mononuclears, 39.2 per cent.; polynuclears, 24 per cent.; eosinophiles, 1.6 per cent.; myelocytes, 30 per cent.; mast-cells, 0.8 per cent.<br>Small mononuclears, 1.6 per cent.; large mononuclears, 22 per cent.; polynuclears, 21.2 per cent.; eosinophiles, 0.4 per cent.; myelocytes, 54.4 per cent.; mast-cells, 0.4 per cent. |
|  | 42,200 to 36,500 | Myelocytes, 47 per cent.; polynuclear neutrophiles, 32 per cent.; lymphocytes, 14 per cent. |

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(6) *Hirschfeld* and *Alexander :* Berl. klin. Woch., 1902, No. 11, p. 231.
(7) *Michaelis :* Zeit. f. klin. Med. 1902, vol. xlv, p. 87.
(8) Am. Jour. Med. Sci. 1903, vol. cxxvi, p. 375.
(9) *Türk :* Cent. f. innere Medicin, 1903, No. 13, p. 336.

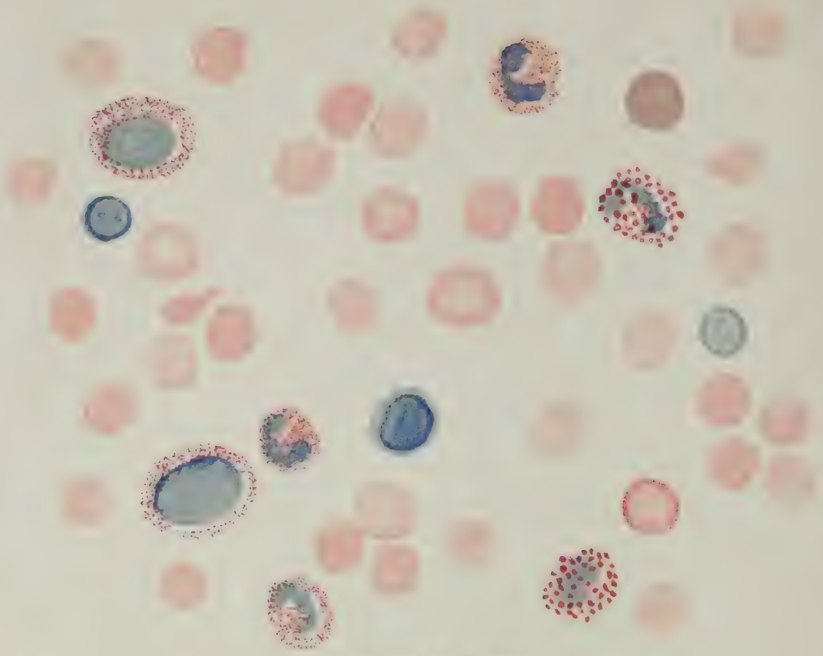
oral mucosa, a finding which suggests the tonsillar ulceration occasionally seen in acute lymphatic leukæmia. The diagnosis of an acute myelogenous leukæmia must rest almost entirely on a marked increase in the white cells of the blood, showing a large proportion of myelocytes and accompanied by a marked anæmia. A differential diagnosis between a leucocytosis and acute leukæmia is difficult, unless the white cells are above fifty or one hundred thousand, and some of the cases above might be explained as a high leucocytosis in the course of an infection, as but few of the reports record bacteriological examinations.

### CHRONIC MYELOGENOUS LEUKÆMIA

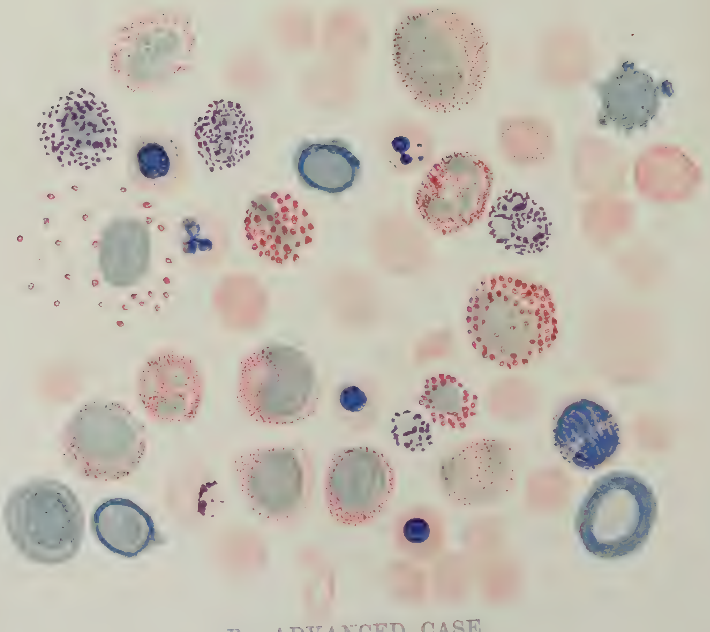
The disease, as a rule, is very chronic in its course, one case in the writer's experience giving marked symptoms seven years before death, while there are many recorded in which the disease lasted from one to three years.

The spleen is usually enormously enlarged, the lymph nodes and the liver may also be increased in size, and leukæmic infiltrations may exist in the organs and the skin. The same infiltrations with hæmorrhages may occur in the retina. The blood shows profound changes both in the red and the white cells.

The **red cells** may be nearly up to the normal number or they may be reduced below a million. They show, as a rule, the changes seen in secondary anæmia; that is, a reduction of the hæmoglobin to a greater degree than the reduction of the red cells, and a moderate number of nucleated reds of the normoblastic type with some poikilocytosis. On the other hand, cases are not rare in which the changes are those which one expects to see in the most severe pernicious anæmia—a high hæmoglobin index, numerous megalocytes and megaloblasts, the latter rarely containing mitotic figures. The red cells show marked polychromatophilia and granular degeneration, both in the nucleated and non-nucleated varieties. The cells are often vacuolated and give other evidences of degeneration in the abundant poikilocytosis usually present. The question of the determination of the hæmoglobin and the hæmoglobin index in cases with a high leucocyte count, can not always be answered satisfactorily, as the opacity of the mixture renders difficult an accurate reading of the hæmoglobinometer. If the Fleischl-Miescher apparatus be used, the hæmoglobin can be accu-



A.—EARLY CASE.



B.—ADVANCED CASE.

PLATE VIII.—MYELOGENOUS LEUKÆMIA.

# DESCRIPTION OF PLATE VIII

*Stained by the Jenner Method*

### *Figure A.*—Early Case of Myelogenous Leukæmia

The red cells in an early case of myelogenous leukæmia show very few changes, but may show slightly irregular staining. A few poikilocytes and an occasional cell with polychromatophilia and granular degeneration are seen, but nucleated red corpuscles are not very abundant in the early stage. The presence of a considerable number of neutrophile myelocytes combined with a marked leucocytosis is the only alteration in the blood which is of diagnostic importance.

### *Figure B.*—Advanced Case of Myelogenous Leukæmia

The red cells show moderate anæmia with some poikilocytosis, but, as a rule, changes in the erythrocytes are not nearly so severe as in a case of anæmia of similar clinical gravity. Nucleated red cells of a normoblastic type, however, are exceedingly frequent, and mitotic figures may usually be demonstrated in an advanced case. The cell body of the red corpuscles during mitosis often takes a deep blue stain and contains numerous basophile granules. The white cells show every imaginable variation from the normal. The polynuclear neutrophiles are often small and the granules imperfectly formed. The myelocytes may contain only a few granules or none at all, or the granules may stain irregularly. Occasionally nucleoli may be seen in the nucleus of the neutrophile myelocyte. The basophile cells are very abundant and usually polynuclear; occasionally, however, they are mononuclear. Lymphocytes of all varieties are seen in moderate numbers, and sometimes it is exceedingly difficult to separate them from the myelocytes without granules, the cell body of the latter staining in the same manner as the cell body of the lymphocyte. The cells of an indeterminate type are frequently seen and have been considered as nucleated red cells with marked polychromatophilia, or as primary bone marrow cells (myeloblasts), or as the irritation forms of Türk. Such a cell is figured in the lower right-hand corner of the plate.

rately determined by centrifuging the blood after dilution and removing the clear supernatant fluid to the cells with a pipette. The Dare instrument also gives fairly satisfactory results. A determination of the total iron content of the blood by Jolles' method may also furnish satisfactory results.

The **white cells** in myelogenous leukæmia are greatly increased, except in very mild cases. The average may be assumed as from one hundred thousand to two hundred thousand in the early cases, up to an upper limit of about one million two hundred thousand. During the remissions of the disease, or under arsenic treatment, the leucocyte count may be normal, but careful examination will usually show the presence of myelocytes. The motility of the normally actively amœboid polynuclears is diminished; the myelocytes occasionally show a very slight motility; but not as much as is seen in the myelocytes in the normal bone marrow.

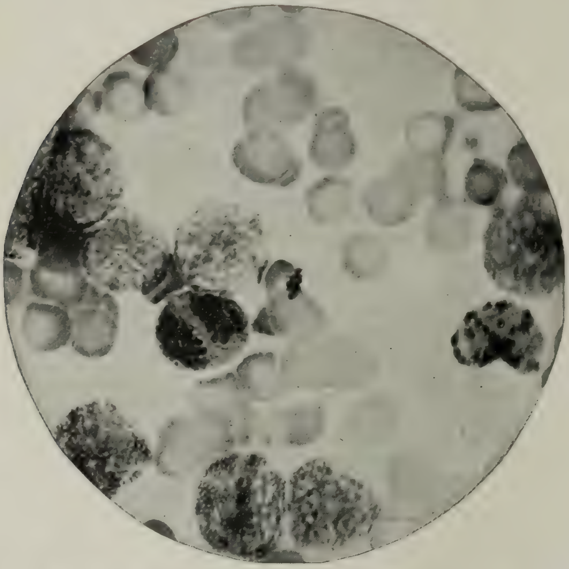


FIG. 45.—MITOSIS IN RED CELL. MYELOGENOUS LEUKÆMIA. EARLY STAGE AFTER SEPARATION OF CHROMOSOMES.

The polynuclear neutrophiles are always positively increased; that is, they are always more numerous to the cubic millimeter than in normal blood, and may reach as high as two hundred thousand or over. They vary much more in size than in normal blood, measuring from 5 to 15 micra. The neutrophile granules may be absent or very scarce in stained preparations; the granules may be free in the serum or clustered about a very faintly staining nucleus without visible cell body. The size and staining reactions of the individual granules also vary, this being best marked in the Jenner preparations.

The polynuclear eosinophile cells are increased in number. They present no marked differences from those found in normal blood. The large mononuclears with their well-staining nuclei and pale cell bodies are often increased, but are not to be confused with the large unripe myelocytes with basophilic protoplasm and without distinct granulations, which are so abundant in very chronic cases. Cells with basophilic granules and polymorphic nuclei are quite constantly found in considerable numbers; they may even rise to 150,000 to the cubic millimeter. The granules possess the irregularity of shape and size seen in normal blood, and with thionin and with methylene blue or Jenner stain show a peculiar

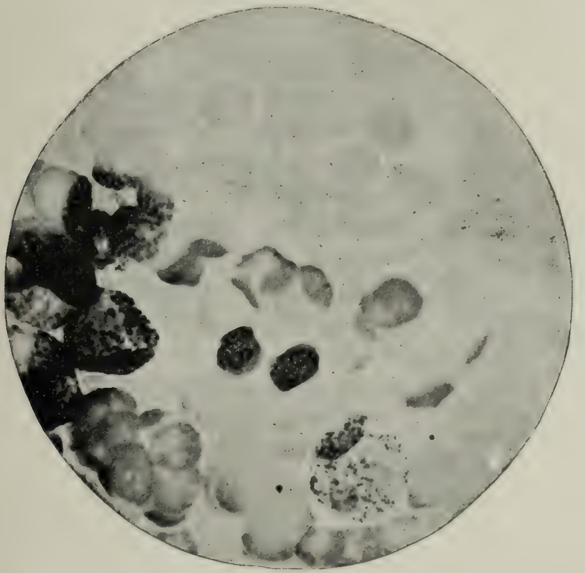


Fig. 46.—Mitosis in Red Cell. Myelogenous Leukæmia. Late Stage, only a Bridge of Cell Body Remains Between the Nuclei.

metachromatism noted in these cells in normal blood; that is, they stain of a deep purple color with these dyes, whereas the nucleus of these and other cells stains a pure blue.

The lymphocytes, both large and small, are quite variable. They may be moderately increased or remain normal. In some cases the large forms are especially abundant and show the characteristic ear-like projections of the narrow basophilic cell body, so well seen in lymphatic leukæmias.

The myelocytes are, so to speak, the characteristic lesion in the blood, and without their presence a diagnosis of myelogenous leukæmia can not be made. They are of three types, neutrophilic, eosinophilic, and basophilic, the last being much less common than the other two. The neutrophilic forms are the most abundant and the most variable in type. They have a large oval nucleus, usually eccentric, and neutrophile granules in varying quantities, at times none at all. The cell body in these cases is faintly stained with basic dyes as a rather coarse reticulum. These are the early forms seen in bone marrow under normal conditions and called myeloblasts by Naegeli, or ancestral cells by Grawitz.

The neutrophilic myelocytes sometimes reach an extraordinary size, even 13 micra in diameter. Degenerated forms are common in advanced leukæmias. They may show only a wide scattering of the granules, or the cell body may be vacuolated, or small tufts may protrude from the cell outline, and these tufts may be cast off and appear free in the protoplasm. Some of the myelocytes may have two nuclei arranged at different poles of the cell, a probable evidence of cell division without separation of the cell body. Active mitotic division is a great rarity in the peripheral circulation, but may be observed in the bone marrow and in the vessels of the liver and other organs in some abundance; apparently the physical conditions in the rapidly moving blood are not conducive to cell division.

The eosinophilic myelocytes are quite characteristic of leukæmia, though not invariably found in large numbers, and though they may rarely be seen in other diseases. A few cases are on record in which no eosinophile myelocytes were present in the blood.[1] They are not, however, found as often as the neutrophile forms in the blood of diseases other than leukæmia. Occasionally the eosinophile cells may contain both eosinophile and basophile granules; but these do not appear to be the same as the basophile granules seen in the basophile cells, for they do not stain with the peculiar metachromatism mentioned above, but take a pure blue, like the nucleus. Degenerate forms are frequently seen with faintly staining nuclei and widely scattered granules.

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[1] *Strauss* u. *Rohnstein:* Blutzusammensetzung bei den versch. Anämieen, 1900, p. 142. *Simon:* Am. Jour. Med. Sciences, 1903, vol. cxxv, p. 984.

The basophilic myelocytes have the same general features as the eosinophilic, except that they are rather rarely found, and the granules show the same irregularity in shape and size that they do in the polynuclear forms. The individual granules often stain more deeply at the periphery than at the center, giving the impression that they are mere stained rings lying in the cell body. The cells which are characteristic of the disease—that is, the myelocytes—are found in large numbers in the organs of the body, especially in the spleen, lymph nodes, and liver. In the spleen and lymph nodes mitotic figures are often abundant in the myelocytes, suggesting the possibility that these cells are deposited from the blood, and, continuing to grow, form fresh centers of production and distribution similar to the metastases of a malignant tumor. The myelocytes, with this exception, do not leave the circulation in response to the ordinary chemotactic agents. In acute pneumonias in leukæmic patients, the cells of the sputum are polynuclear, as are also the cells from pus. In the fluid from serous exudates into the pleura and abdomen, however, a few myelocytes and basophile cells are occasionally seen, the conditions governing the exudation seeming to be different. During acute infections [1] the myelocytes may be greatly reduced in numbers in the circulation, and may even entirely disappear as far as can be determined from the examination of smears of the blood. If the infection is long continued the deposits of myelocytes in the organs may be reduced or disappear also, which is shown clinically by a reduction in the size of the liver and spleen. The same series of phenomena can often be observed after treatment with arsenic and also just before death.

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[1] *E. Weil:* Les infections et la leucemie, Gaz. hebd., 1900, p. 829. *Dock:* Influence of Complicating Diseases upon Leukæmia, Amer. Journal Med. Sciences, 1904, p. 563.

## UNDETERMINED TYPES OF BLOOD DISEASE

### FATAL ANÆMIA WITHOUT CHARACTERISTIC LESIONS

A moderate number of cases [1] of rapidly fatal anæmia without hyperplasia of the bone marrow have been described, attention having been first called to the condition by Ehrlich.[2] The symptoms of the disease are a rapidly progressing anæmia, often accompanied by hæmorrhages into the mucous membranes and without the changes in the blood which are usually considered to accompany pernicious anæmia.

The hæmoglobin is reduced in direct proportion to the reduction of the red cells, which are usually of normal size. Poikilocytosis and anisocytosis are not as well marked as is usual in so severe an anæmia. Nucleated red cells are either not found or are exceedingly scanty.

The leucocytes in most of the cases have not been increased in number and show usually a relative lymphocytosis. The autopsy findings have been negative as far as the blood-making organs are concerned.

The bone marrow does not show marked hyperplasia, but is fatty even in situations where it is reddish in normal individuals. Ehrlich has classed these cases as aplastic anæmias, and thinks that the bone marrow has been unable to respond to the rapid destruction of the red cells which has taken place in the body.

In a case of the writer's the red cells fell from 1,600,000 to 790,000 in two weeks. The leucocytes, which were at first 45,000 with 81 per cent. of lymphocytes, fell to 16,000 with 39 per cent. lymphocytes on the day before death. On autopsy the heart was fatty and the spleen moderately enlarged. Both the renal vein and the renal artery of the left kidney were plugged by a firm thrombus, and the kidney substance was anæmic and had lost all structure. The bone marrow was yellow throughout, even in the spongy tissue of the sternum and the vertebræ.

Cases of this type are difficult to classify, and further observations must be made before the definite position of the disease can be determined.

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[1] *Lipowski* u. *Engel:* Zeit. f. klin. Med., 1900, Bd. xl, p. 17. *Schauman:* Sammlung klin. Vorträge, No. 287, p. 234. *Bloch:* Beit. v. Ziegler, 1903, Bd. xxxiv, p. 347.

[2] Charité Annalen, 1888, Bd. xiii, p. 300.

### FORMS INTERMEDIATE BETWEEN PERNICIOUS ANÆMIA AND LEUKÆMIA

In another group of obscure cases the red cells show the changes which are considered characteristic of pernicious anæmia, while the leucocytes either remain low and show a large proportion of myelocytes or lymphocytes, or they rise to the limits which we are accustomed to consider as characteristic of leukæmia. The present tendency is to class these conditions as of a leukæmic nature. Among the first of these cases to be reported was that of Litten,[1] in which a person with pernicious anæmia gradually developed symptoms of a myelogenous leukæmia. As the examination of the blood had not then reached very great development, it is possible that this case may be classed as a myelogenous leukæmia, which first came under observation during a remission of the process with a reduction in the number of leucocytes, and was therefore considered as primarily an anæmia.

Other cases, for example that of Körmöczi,[2] have shown changes in blood morphology from that considered characteristic of pernicious anæmia to lymphatic leukæmia without the visceral lesions of these two conditions being found at autopsy.

Osler [3] has seen a case of splenic anæmia with a red cell count of 1,000,000, in which the leucocytes increased from normal to a very large number two weeks before death, 84 per cent. being lymphocytes. He considers the increase as indicating the transition to a true leukæmia.

Zypkin [4] reports a similar case in which the increase in the leucocytes was observed only five days before death. The proportions of the leucocytes were as follows: Polynuclear neutrophiles, 12 per cent.; lymphocytes, 71 per cent.; myelocytes, 2.4 per cent.; mononuclear cells, with granulations, 14.4 per cent.; eosinophiles, .3 per cent. The red corpuscles fell to 1,550,000 with

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[1] Berl. klin. Woch., 1877, pp. 257, 278.

[2] Deut. med. Woch., 1899, p. 238.

[3] Amer. Journ. Med. Sci., 1900, vol. cxix, p. 69.

[4] Wien. klin. Woch., 1903, p. 1085.

24 per cent. of hæmoglobin; i. e., a high index. Megaloblasts and normoblasts were abundant. The bone marrow was hyperplastic.

A case of lymphatic leukæmia, which may serve as a type of the intermediate group between pernicious anæmia and leukæmia, has been recently published by Hitschmann and Lehndorff.[1] The patient was under observation for about three weeks with a continuous temperature, enlarged lymph nodes, and normal sized spleen. There was an exanthematous eruption over the body which partially disappeared under pressure and also a petechial rash, especially abundant on the extremities. (A similar eruption, resembling a maculo-papular syphilide, was observed by Mannaberg and Spiegler[2] in a case of acute leukæmia.) The blood changes noted were rapidly increasing anæmia, the red cells diminishing from 2,100,000 to 724,000 in three weeks. A large number of megaloblasts were present, rising at the termination of the disease to about ten thousand to the cubic millimeter. The hæmoglobin index was high. The white cells at the beginning of the disease were 10,500 and increased to 35,000 toward the termination. The differential proportions of the white cells at the beginning of the disease were as follows: Neutrophiles, 4 per cent.; eosinophiles, 5.7 per cent.; small lymphocytes, 44.4 per cent.; large mononuclear cells, 45.9 per cent. Before death the number of neutrophiles and eosinophiles fell to 5 per cent. and the mononuclears and lymphocytes increased to 95 per cent. Of these 19 per cent. were small lymphocytes and 76 per cent. large mononuclears. At the same time a considerable number of large basophile, non-granular cells appeared in the circulation, of the type usually found in the bone marrow.

### LEUKANÆMIA

An observation of von Leube's has recently caused renewed interest in the study of the leukæmic anæmias.[3] The subject was a ten-year-old boy, who suffered apparently from a combination of pernicious anæmia and myelogenous leukæmia, running a very acute course of about three weeks with clinical symptoms of an acute infectious disease with hæmorrhages. The red cells were reduced to 256,000 to the cubic millimeter; numerous nucleated reds were present, some 70 per cent. of which were of the megaloblastic type; while the white cells numbered 10,000. The differential count showed 40 per cent. lymphocytes, 44 per cent. polynuclear neutrophiles, 13 per cent. neutrophile myelocytes, .6 per cent. eosinophile myelocytes, and 2 per cent. large mononuclear leucocytes. The bone marrow was hyperplastic and red, with myeloid changes in the spleen and liver, but no deposits of iron in the organs. The name of leukanæmia has been suggested by von Leube for this condition.

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[1] Zeit. f. Heilkunde, 1903, Bd. xxiv, p. 190.

[2] Gesells. f. innere Med. Wien, Jan. 9, 1902.

[3] Deut. Arch. f. klin. Med., 1901, Bd. lxix, p. 331. See also *Hirschfeld*; Ueber atypische Leukämieen. Folia hæmatologica, 1904, Bd. i, p. 150.

Luce [1] has recently described a somewhat similar case in which the white cells were at no time increased over 81,000. The red cells were 1,200,000 and the hæmoglobin 30 per cent. The differential count showed 46 per cent. polynuclear neutrophiles, 49 per cent. large lymphocytes and non-granular myelocytes, and 4 per cent. of small lymphocytes. The autopsy showed leukæmic infiltration of the liver and kidney and absence of deposits of iron in the organs.

A case of von Jaksch [2] seems related on the one hand to acute myelogenous leukæmia, and on the other to an acute infectious process. The patient had a marked anæmia with multiple areas of periostitis, and enlargement of the liver, spleen, and lymph nodes. The leucocytes varied between 10,000 and 70,000, from 4 to 30 per cent. of neutrophile myelocytes being present. There is no evidence that the disease could not be considered an irregular type of myelogenous leukæmia, the blood picture being altered by the acute periostitis, a phenomenon often observed in typical cases of myelogenous leukæmia complicated by an acute infectious process.

Transitional cases in which the blood changed from the lymphatic type to the myelogenous have been reported,[3] but such alterations are often seen only a few days before death, and are doubtless agonal and not accompanied by corresponding changes in the blood-making organs.

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[1] Deut. Arch. f. klin. Med., 1903, Bd. lxxvii, p. 215.

[2] Zeit. f. Heilkunde, 1901, N. F., Bd. ii, p. 259.

[3] *Fowler:* Internat. Clinics, 1903, Thirteenth Series, vol. iii, p. 217. *Wilkinson:* Lancet, 1903, p. 1739.

## FORMS INTERMEDIATE BETWEEN PSEUDO-LEUKÆMIA AND LYMPHATIC LEUKÆMIA

A change in the morphology of the blood from that characteristic of pseudo-leukæmia to the leukæmic type is not, as far as our knowledge extends at present, accompanied by the development of a new disease, but much more probably depends upon the extension of the local hyperplasia of the lymph nodes to the lymphoid tissue in the spleen and bone marrow. In these organs the anatomical relations of the vessels to the lymph cells is so intimate that the latter may easily escape into the circulation and give the blood picture of a lymphatic leukæmia.[1]

Closely related to these intermediate forms are the cases of general sarcomatosis accompanied by the blood changes of lymphatic leukæmia,[2] and those of chloroma[3] in which the blood may show an excess of lymphocytes or an excess of marrow cells of either the myeloblastic or the myelocytic type. It is probable that the blood changes are not produced until the new growth invades the marrow, and either sets free in this locality the characteristic tumor cells or incites an active hyperplasia of the marrow cells, such as is seen in cases of carcinosis of the bone marrow with the blood picture of myelogenous leukæmia.

## DIAGNOSIS OF INTERMEDIATE TYPES OF BLOOD DISEASE

It is evident that our knowledge of the subject of blood diseases is still very incomplete, and diagnostic conclusions from the morphology of the blood should always be controlled by the results of the clinical examination of the patient. By this the writer does not mean to state that a myelogenous or lymphatic leukæmia or a pernicious anæmia can not be diagnosed by the morphology of the blood; on the contrary, the blood may often indicate the disease when a diagnosis can not be made by the clinical study of the case. In the typical cases, however, the blood remains comparatively constant in its morphology and except during periods of marked improvement the diagnosis can be made at a glance.

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[1] For an excellent review, see *Türk:* Wien. klin. Woch., 1903, p. 1073.

[2] *G. Lazarus:* Multiple Sarcome mit perniciöser Anämie und gleichzeitiger Leukämie. Dissertation, Berlin, 1890. *Strauss:* Sarcomatose u. Lymph. Leukämie. Charité Annalen, xxiii, 1898, p. 343. *P. Grawitz:* Deut. med. Woch., 1890, p. 458.

[3] *Dock* and *Warthin:* Medical News, vol. lxxxv, 1904, p. 971.

In the obscure cases which we have just considered one immediately notices that there are either great fluctuations in the counts and morphology or that the changes appeared a few days before death. A diagnosis of the anatomical conditions accompanying the disease should not be made under these circumstances from the blood alone.

## VI. GENERAL PATHOLOGY OF THE BLOOD

### BLOOD CHANGES IN SURGICAL CONDITIONS

As mentioned under the heading of leucocytosis, the administration of an anæsthetic is followed by a slight rise in the number of leucocytes. The same phenomenon follows the giving of a saline infusion. A moderate loss of blood will cause a rise in the leucocytes above normal for twenty-four hours or more, depending upon the amount of blood lost. Shock also causes a moderate rise unless very severe, when the leucocytes are diminished.

Pus anywhere in the body causes a leucocytosis, except when it is thickly encapsulated or the infection is one of extraordinary severity. The so-called pus from a cold abscess is only débris, not pus in a true sense, and does not give rise to a leucocytosis. The thick purulent fluid obtained from cases of pyosalpinx is very generally sterile, so that the great majority of such patients show no leucocytosis.

Catarrhal appendicitis, in the writer's experience, generally gives a leucocytosis of 10,000 to 15,000, but not invariably, while in perforate appendicitis and general peritonitis a leucocytosis is usually present in the early stages of the disease, but as the condition becomes more severe and the patient's resistance fails, the leucocytes fall gradually. In a good many cases of catarrhal appendicitis, however, the leucocytosis may be quite transient so that the general condition of the patient is of more importance than the height of the leucocyte count in judging of the need of operative interference.

If pus is formed in small amounts and is well shut off from the general peritoneal cavity, the leucocytes may not rise or the increase may be so slight that it hardly exceeds the normal maximum of 10,000. Under these circumstances the presence of an iodophilic reaction in the polynuclears is of great value in determining the presence of an exudate.

The number of leucocytes gives no clue to the amount of pus formed, but a safe general rule is that the small exudates or beginning peritonitis give high leucocytoses, large exudates a high leucocytosis, generally falling as the process becomes more widely spread, while the gangrenous and perforating cases show a low leucocytosis. When in doubt, the pulse, facies, and general condition should always outweigh the leucocyte count in deciding on operative procedures.

With low counts a differential enumeration of the leucocytes should always be made. If the polynuclears form 85 per cent. to 95 per cent. of the whole, a severe sepsis is probable, while if the lymphocytes are high, typhoid fever should always be thought of. Bloodgood, Curschmann, and others, consider a leucocytosis of 20,000 to 25,000 as indicating the necessity of an immediate operation, though such cases have been known to recover spontaneously.

If the leucocytes do not return to normal in a day or so it is probable that there are other foci of pus which have not been drained, that a general peritonitis is beginning, or that a fæcal fistula has formed. Revision of the wound should therefore be promptly carried out.[1]

## BLOOD CHANGES IN GENERAL DISEASES

### TYPHOID FEVER

The blood in typhoid fever, aside from the agglutination reaction, shows peculiar changes in the numerical relations of the leucocytes, which are sometimes so well marked as to permit of a probable diagnosis being made from the morphology during the second and third weeks. The different stages of the disease afford different blood pictures. During the first week some observers have stated there is a moderate leucocytosis,[2] which quickly disappears and is followed by an absolute and relative diminution in the number of neutrophiles, but Higley[1] in a number of early cases was unable to demonstrate an initial rise in neutrophiles and found instead a leucopenia with a relatively high lymphocyte count. My own observations on a few patients who were undoubtedly in the first week of the disease agree with those of Higley. In the second week there is a further diminution in the number of neutrophiles, and toward the latter part of the week a rise in the number of lymphocytes, with practically a complete absence of eosinophiles. At this time, therefore, a probable diagnosis of typhoid fever can be made on an adult with a continued fever, a diminished leucocyte count, a relative increase in the number of lymphocytes, and a diminution in the number of eosinophiles. In children the changes are not so well marked either in the relations of the leucocytes to each other or in the diminution in their absolute number.

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[1] For further details on this subject, see:—*Bloodgood:* American Medicine, 1901, vol. i, p. 306. *Curschmann:* Münch. med. Woch., 1901, pp. 1907, 1962. *Gerngross:* ibid., 1903, p. 1586. *Goetjes:* ibid., 1903, p. 723. *Federmann:* Mitth. a. d. Grenzgebieten d. Med. u. Chir., 1903, Bd. xii, p. 213.

[2] *Thayer:* Johns Hopkins Hosp. Reports, 1900, vol. viii, p. 487.

In the third week the relative increase of the lymphocytes becomes still more marked, the neutrophiles go still lower, and the eosinophiles begin toward the end of the week to increase slowly.

One case seen by the writer may be quoted as an example of the profound alterations in the proportion of the leucocytes in the third week. The leucocyte count was 5,000. Polynuclear neutrophiles were present in a proportion of 33 per cent.; large lymphocytes, 47 per cent.; small lymphocytes, 20 per cent. No basophile or eosinophile cells were seen in the 500 leucocytes counted. The Widal reaction was positive at 1 to 200 within an hour.

In some cases in adults, however, the lymphocytes are not much increased beyond the normal number of 1,500 to the cubic millimeter, even at this period.

During the fourth week the neutrophiles reach their lowest point, the lymphocytes are often more abundant than the neutrophiles, while the eosinophiles slowly increase toward their normal percentage. As soon as the fever disappears the neutrophiles begin to increase and gradually rise to the normal, the eosinophiles often increase quite beyond the average 4 per cent. and remain so for several months, the lymphocytes slowly diminish.

Complications caused by infections by bacilli other than typhoid cause only a slight rise in the number of polynuclear neutrophiles, far less than would appear with a similar infection not complicated by typhoid. The absence of leucocytosis, in spite of a severe complicating infection, is a sign of bad prognosis. In uncomplicated cases a moderate diminution of the neutrophiles and eosinophiles, and a well-marked rise in the lymphocytes, is of good prognosis. A hæmorrhage or perforation of the intestine usually causes a rise in the leucocytes, but occasionally in fatal cases the leucocytes may fall after a perforation.

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[1] Proc. New York Path. Soc., 1903, vol. iii, N. S., p. 87.

The diminution of the neutrophiles and eosinophiles in the course of typhoid fever is due probably to the action of the toxins of the disease on the bone marrow, reducing its ability to produce leucocytes in normal numbers, while the action of the toxin on the lymphatic apparatus is not so extreme, thus permitting a relatively large number of lymphocytes to appear in the blood.[1]

In typhoid fever the existence of a leucocytosis points to some infection other than typhoid, probably a pneumonia, phlebitis, or a perforation. There is occasionally a leucocytosis in intestinal obstruction, coming on soon after the onset of the condition, but high counts point to gangrene. Tuberculous meningitis, it must be remembered, may occasionally give a leucocytosis, which is contrary to the general rule that tuberculous conditions do not cause an increase in the peripheral leucocytes. In cholelithiasis a leucocytosis indicates some acute lesion of the gall bladder or ducts, as the presence or passage of gall-stones does not give rise to a leucocytosis.

### PNEUMONIA

The blood in this disease is quite regularly characterized by the presence during the attack of a marked polynuclear leucocytosis, the average number being about 25,000 in well-marked cases. The rise begins with the time of the initial chill, while the fall coincides with the cessation of the active process in the lung.

In mild cases the leucocytosis may be moderate, but in those in which the reaction of the body to the infection is vigorous a marked leucocytosis is the rule. In severe infections with slight reaction, the leucocytosis is apt to be low. This is generally considered a symptom of bad prognosis.

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[1] For a fuller discussion of this subject, see:—*Naegeli:* Deut. Arch. f. klin. Med., 1900, Bd. lxvii, p. 279. *Kast* u. *Gütig:* Deut. Arch. f. klin. Med., 1904, Bd. lxxx, p. 105.

The eosinophiles and the lymphocytes are reduced during the course of the leucocytosis, but after the crisis they are often slightly increased and a few myelocytes may be present. The temperature and the amount of lung tissue invaded have no quantitative relations to the leucocytosis. The temperature may be high and the amount of lung involved very great, with a low leucocyte count.

### DIPHTHERIA

There is a very slight reduction of the red cells and of the hæmoglobin during the course of a diphtheritic infection, though occasionally a polycythæmia has been noted. Cases treated with the specific serum as a rule show a temporary hypoleucocytosis following the injection, while the diminution in the red cells and hæmoglobin is not so marked as in the untreated cases. In cases of moderate severity the leucocytes are increased during the height of the disease. The cases in which there is no such increase may be either very severe, in which event the infection has inhibited the leucocytosis, as is seen in pneumonia, or very mild.[1] The increase in the leucocytes is largely due to an increase in the polynuclear cells. In many severe cases of diphtheria, but not in all, myelocytes and "irritation forms" have been found, occasionally rising as high as 14 per cent.[2] The glycogen reaction is usually, but not always, present.

### SCARLET FEVER

Nearly all cases show a leucocytosis of from ten to forty thousand with a relative increase in the number of polynuclears. The rise in the white cells is parallel to the curve of the temperature, with the maximum at the height of the rash.[3]

About the fourth day of the disease there is usually a rise in the eosinophiles, occasionally as high as 15 per cent., and this increase continues after the temperature has fallen.[4] The chief value of the blood examination in this disease is to furnish a differential diagnosis between mild cases without pronounced rash and measles, in which no leucocytosis is present.

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[1] *Ewing:* Clinical Path. of the Blood, New York, 1903.

[2] *Engel:* Deut. med. Woch., 1897, p. 118.

[3] *Reckzeh:* Zeit. f. klin. Med., 1902, Bd. xlv, pp. 107 and 201.

[4] *Sacquepée:* Arch. de Méd. exp., 1902, p. 101. *J. van den Berg:* Blutuntersuchungen bei Scharlach. Diss. Freiburg, 1898.

### MEASLES

As stated in the last paragraph, the blood changes in measles are very slight. Usually there is no leucocytosis, but quite frequently a diminution in the number of polynuclears during the stage of eruption. The eosinophiles in measles—in contrast to scarlet fever—are diminished. In German measles the condition is the same.

### VARIOLA

In variola there is usually a moderate diminution in the number of red corpuscles and in the hæmoglobin during the course of the disease, and after the appearance of the vesicles an increase in the leucocytes.

The most important phenomenon, and one which is occasionally of diagnostic value in differentiating variola from other acute infectious diseases with skin lesions, is the appearance in the blood of a large number of mononuclear cells; of these cells small lymphocytes form 30 to 40 per cent., myelocytes usually from 2 to 10 per cent., though in very severe cases, especially with hæmorrhages, there may be 25 per cent. of myelocytes in the blood. The "irritation forms" of Türk form from 2 to 10 per cent.[1] These changes are most marked in the cases with abundant pustules containing pus and streptococci.[2] Nucleated red cells are occasionally seen, especially in the hæmorrhagic cases.

### VACCINIA

Vaccinia in children occasionally produces a slight leucocytosis. In adults no changes have been noted, unless the eruption is generalized, when there may be a moderate leucocytosis.

### CHICKEN-POX

In the slight cases no changes have been noted. The more severe types show a moderate leucocytosis and occasionally a few myelocytes are present.[3]

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[1] *Weil:* Soc. de Biol., 1900, p. 615.

[2] *Courmont* et *Montagard:* Journ. de Phys. et de Path. gén., 1900, p. 557.

[3] *Nobecourt* et *Merklen:* Journ. de Phys. et de Path. gén., 1901, p. 439.

### PERTUSSIS

The proportions of the leucocytes in this disease show a constant and characteristic change. During the course of the disease, especially in young children, there is a marked leucocytosis with a relative increase in the lymphocytes. No such change has been found by Meunier[1] in uncomplicated cases of bronchitis, and the blood examination may therefore afford results of diagnostic value. Occasionally the leucocytes are increased to such an extent that suspicion may be aroused that the disease is of a leukæmic nature, especially if the child shows enlargement of the spleen and lymph nodes as is frequently the case in children with rickets.

Cabot[2] saw two cases of pneumonia during pertussis in which the leucocytes rose to 94,000 to the cubic millimeter, with 69 and 65 per cent. of lymphocytes. A leucocyte count of 236,000 with 65 per cent. of lymphocytes has been reported by Steven.[3]

### ACUTE ARTICULAR RHEUMATISM

A high leucocytosis may occur in acute cases, when a number of joints are involved, while in subacute cases, or those confined to a single articulation, the increase in the white cells may be very slight. During the course of the disease a moderate anæmia usually develops. Since the same phenomena also occur in gonorrhœal, strepto- and staphylococcus arthritis, the blood changes are not of diagnostic value.

### ERYSIPELAS

There is a moderate polynuclear leucocytosis running parallel to the temperature curve, but no other change of importance. Myelocytes are rarely seen and are not of diagnostic or prognostic value.

### SEPTICÆMIA

Generalized infections with the pyogenic cocci regularly incite a marked increase in the leucocytes with a rise to 30,000, 40,000, or even 50,000 cells to the cubic millimeter, though occasionally very rapid and fatal cases occur in which no leucocytosis is found. In these conditions it is probable that the infection has so affected the bone marrow that leucocytes are not produced in normal numbers. In obscure cases the isolation of the infectious agent from the blood is necessary for a positive diagnosis. In distinguishing sepsis with a low leucocyte count from miliary tuberculosis the results of a differential count may be important, the polynuclears being high in the former, the lymphocytes in the latter.

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[1] Arch. de Médicine des Enfants, 1898, tome i, p. 193.

[2] Clinical Examination of the Blood, New York, 1904.

[3] Lancet, 1902, vol. ii, p. 791.

### MENINGITIS

Acute meningitis due to the pyogenic cocci, to the pneumococcus, or to the meningococcus, is usually accompanied by a leucocytosis. Tuberculosis of the serous membranes of the brain and cord also gives rise to a leucocytosis. These facts are occasionally of value in distinguishing meningitis from other diseases which may give similar symptoms; for example, cerebral tumors, cerebral type of lead-poisoning, uræmia, apoplexy, and diabetic coma. Thus cases of typhoid fever with marked cerebral symptoms show a low leucocytosis with a relative increase in the lymphocytes in contrast to the polynuclear leucocytosis of meningitis.

### TUBERCULOSIS

General miliary tuberculosis, as a rule, gives no leucocytosis. In a case reported by Warthin [1] there was a constant leucopenia for a month before death, the white cells diminishing at one time to 600 to the cubic millimeter; but in a few of Cabot's cases [2] there was a moderate leucocytosis. In the writer's experience leucopenia is the rule. In tuberculous peritonitis a leucocytosis is frequently seen.

### SYPHILIS

The most striking phenomenon in this disease is the rapid reduction in the number of red cells beginning soon after the appearance of the initial lesion. The anæmia is generally of the chlorotic type, but in several untreated cases seen by the writer there was a very extreme anæmia, one with only 1,400,000 cells to the cubic millimeter and numerous nucleated reds, including a few megaloblasts. Anæmia of this type has occasionally been mistaken for pernicious anæmia, but the low hæmoglobin index and the clinical features of the case should make the diagnosis.

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[1] Medical News, 1896, vol. lxviii, p. 89.

[2] Clinical Examination of the Blood, 5th ed., 1904, p. 299.

The effect of the treatment is a slow recovery in the number of cells and in the hæmoglobin. The claim which Justus makes that a constant and rapid reduction of the hæmoglobin in untreated cases takes place after the administration of mercury, has not been verified by other observers.[1]

The leucocytes are slightly increased during the stage of eruption and a relative lymphocytosis is not infrequent.

### LEPROSY

In the milder cases there is no constant alteration in the blood, but in persons suffering from an active form of this disease, especially with a fresh nodular eruption, there may be a moderate increase in the eosinophiles. The bacillus of leprosy has been found within the leucocytes in a considerable number of cases.

### HEART DISEASE

In mycotic endocarditis a marked anæmia rapidly develops and a high leucocytosis is usually present. Cases of acute rheumatic endocarditis also show a moderate leucocytosis without marked anæmia. In the chronic stages of heart disease the chief point of interest in the blood is the polycythæmia which frequently exists. Under these conditions, persons who are evidently anæmic give high counts of 5,000,000 red cells or more, with normal or slightly increased leucocytes, and yet when the blood is examined in stained preparations the pale centers of the red cells immediately call attention to the existing anæmia.

The writer has recently seen a case of this type the subject of myocarditis with chronic bronchitis and emphysema, in which not only was there a marked anæmia, but an abundant granular degeneration of the red cells, and a blood count of 7,660,000 red cells with 90 per cent. of hæmoglobin (Fleischl-Miescher) and many normoblasts. The red cells fell to 6,510,000 in three hours after oxygen inhalations.

In congenital heart disease there is almost constantly a very extreme polycythæmia with high hæmoglobin percentages and specific gravity, and a moderate increase in the white cells.

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[1] *Jones:* N. Y. Med. Journ., 1900, vol. lxxi, p. 513.

An interesting case with a very high count is that reported by Kovács[1] of 9,600,000 red cells in a girl of eighteen years of age. The freezing point of the blood was —.69° C. The number of corpuscles was reduced by the inhalation of oxygen to 7,400,000; the freezing point to — .65° C. The reduction in the number of red cells by the inhalation is of especial interest, as it furnishes an explanation of the phenomenon often seen in heart cases where the red cells become approximately normal as the heart compensation is restored by suitable treatment.

### NEPHRITIS

The changes which occur in the blood of patients suffering from nephritis depend very largely upon the type of the kidney lesion. In an acute nephritis a more or less severe anæmia and a slight leucocytosis are the only constant phenomena and are of no value from a diagnostic point of view. In chronic parenchymatous nephritis there is quite regularly a marked reduction in the hæmoglobin and in the number of red cells, and this diminution in the corpuscles may occasionally reach a very extreme degree. The usual variations, however, are between three and four millions with a hæmoglobin of 40 to 80 per cent.

A certain number of the patients with extreme pallor of the skin and every symptom of anæmia may show a high red cell count with only a slight diminution in the hæmoglobin. This is due to an artificial polycythæmia which exists, owing probably to alterations in the circulation and enfeeblement of the heart action. Inhalations of oxygen will often reduce the number of red cells in a few hours by 500,000 to 1,000,000.

In chronic diffuse nephritis the changes are very slight until the later stages of the disease when a moderate anæmia appears, but this diminution in the red cells is often masked by the polycythæmia connected with the œdema of the tissues and enfeeblement of the heart action, which complicates the disease.

Occasionally, in both chronic parenchymatous and interstitial nephritis, we see cases in which the anæmia is the most prominent symptom, and the kidney lesion may give rise to so few symptoms that the nephritis is entirely overlooked. The writer has seen two such cases, in which the red cells fell to 1,000,000 and 700,000 respectively, with changes characteristic of a secondary anæmia and a moderate increase in the leucocytes. The marrow of the long bones was fatty. Possibly the case of infarction of the kidney previously mentioned on page 176 may belong to this category. Labbé[1] mentions several cases in which an extreme anæmia existed, even suggesting the pernicious type. In one the red cell count fell to 418,000. The bone marrow was only slightly hyperplastic. Ewing[2] has also called attention to the anæmic type of nephritis. The blood in these cases is not that of true pernicious anæmia. The red cells are pale and, on an average, microcytic. Normoblasts are more abundant than megaloblasts. The hæmoglobin index is below 1. There is no doubt, however, that cases of true pernicious anæmia co-exist with advanced nephritis, but the two diseases are independent of each other.

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[1] *Korányi:* Zeit. f. klin. Med., 1898, Bd. xxxiv, p. 25.

## VII. DISEASES IN WHICH THE BLOOD CONTAINS PARASITES

### MALARIA

The excitant of the disease known clinically as malaria is a protozoon of the large class of animal parasites, the sporozoa, closely related to the coccidia. The special class of the hæmosporidia to which the malarial organism belongs are parasitic animals which feed upon the red corpuscles of the blood and destroy them. The course of development of the species of hæmosporidia which attack the blood of man has been followed through two quite different cycles. One of these cycles is in the blood, spleen, and bone marrow of the human host; the other is a sexual cycle, the different stages of which take place in the intestinal tract of the female of a particular genus of mosquito, Anopheles. So far as our present knowledge extends, the extra-human cycle is confined to mosquitoes of this genus. The cycle in the circulating blood is the one which was first discovered by Laveran,[3] and is the one which is most easily followed clinically.

The malarial organisms in the blood in the different types of malarial fevers can be classified morphologically for the purpose of description into two groups. In one form of the disease the cells capable of sexual reproduction (the so-called gametes or crescentic forms) are quite sharply distinguished from the amœboid forms which simply pass through the asexual cycle as parasites in the red blood corpuscles. In the other form, the gametes are hardly to be distinguished from the adult parasites of the asexual cycle. The members of the first group are known as the parasites of æstivo-autumnal or pernicious malaria, while the second group includes two forms, the parasites of the tertian and the quartan fevers.

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[1] *Labbé* et *Lortat-Jacob :* Bull. et Mém. de la Soc. Anat., No. 7, 1903, p. 553.

[2] Clinical Pathology of the Blood, New York, 1903.

[3] C. R. Acad. de Sci., Paris, 1881, T. xciii, p. 627.

### THE DEVELOPMENT OF THE ÆSTIVO-AUTUMNAL FORM OF PARASITE

The life history of this form of parasite will be considered first, as the morphological differentiation of the gametes from the asexual parasites renders the different stages easier to follow than in the other two groups of malarial fever.

**Asexual Cycle.**—If the fresh blood of a patient suffering from æstivo-autumnal fever be examined about the time of a chill the red cells will be seen to contain very small, highly refractile, actively amœboid bodies, which move about in the cell and appear to lie on its surface or in its substance (Plate IX, Figs. 57, 58, 59, 60, 61). While at rest the plasmodium assumes a signet-ring shape and at this stage contains no pigment. If another examination be made of the blood a few hours later, these small ring forms will be seen to have grown larger and a few very small brown or black pigment granules can be seen, generally on the periphery of the organism (Plate IX, Fig. 62). This pigment—melanin—is produced by the parasite from the hæmoglobin of the red cells and is in the form of fine granules or rods. Its exact chemical composition is not known, but it is probably hæmatin or an allied substance. Under ordinary conditions in man a large number of cycles of the organism are developing simultaneously, so that all the early stages can usually be seen in a single preparation if the organisms are at all abundant.

The red cell during this development of the parasite usually undergoes marked change, best seen in fresh preparations. It shrinks slightly, often becomes crenated, and its natural pale-yellow color frequently deepens to a brassy tint. Dotting of the substance of the red cell, such as is seen so commonly in the cells infected by the tertian parasite, is not frequent in the cases occurring outside of the tropics, but some observers have noted this form of degeneration of the red cell both when infected with gametes and early amœboid forms.[1] The dots are usually much coarser than in tertian malaria.

At this point in the cycle, when the amœboid form occupies about one-fourth of the disk of the red cell, the development of the parasite in the peripheral circulation ceases and is continued in the internal organs, especially the spleen, the bone marrow, and the brain capillaries. In infections of great severity an occasional segmenting form may be met with in the peripheral blood stream, but only very exceptionally. If at this time the spleen be punctured and a smear made of the blood so obtained, many adult and segmenting forms will be found, often included with their enveloping red cell in one of the large macrophages of the splenic tissue. The adult plasmodium thus seen is an actively amœboid body occupying from a fifth to a half of the body of the red cell (Plate IX, Figs. 63, 64, 65).

The pigment is small in amount and the minute granules are peripherally arranged about the edge of the plasmodium. When maturity is reached the pigment collects near the center of the organism, the uniform structure of the plasmodium is altered by the appearance of numerous very small clear dots representing nuclei, which can be stained by suitable procedures, and finally the whole mass, or schizont, segments into spores, or more properly—to follow the systematic nomenclature of the coccidia—merozoites (Plate IX, Fig. 66). The number of these merozoites formed from a single plasmodium in æstivo-autumnal malaria varies from six to thirty; the average is about fifteen. The shriveled remains of the red cell may occasionally be seen around the segmenting forms, but, as a rule, the plasmodium is set free before segmentation takes place. The whole process of growth and division is completed in from twenty-four to forty-eight hours, and the small merozoites set free by the segmentation immediately enter other red corpuscles, as the small amœboid forms mentioned above, and thus complete the asexual cycle.

Christy[2] has been able to trace the steps of the penetration of the red cell by the merozoites, shortly after their formation by

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[1] *Stephens* and *Christophers :* Practical Study of Malaria, London, 1903, p. 37.
[2] Brit. Med. Journal, 1903, September, p. 645.

# DESCRIPTION OF PLATE IX

*Stained with eosin-methylene azure*

### *Figure A.*—Quartan Parasites

**1** to **5.** Small merozoites in the red cells. The chromatin is either spherical, horseshoe shaped, or in two particles.

**6, 7, 8,** and **9.** Gradual growth of the parasite, the formation of pigment, and the breaking up of the nucleus.

**10.** Microgametocyte, early form.

**11.** Macrogamete, early form.

**12, 13, 14,** and **15.** Presegmenting stage of the amœboid parasite.

**16.** Commencing segmentation. The chromatin mass undergoes division and forms a series of small compact masses.

**17.** Early stage of the same process with more complete separation of the chromosomes.

**18.** Pigment collects in the center of the parasite and the chromosomes arrange themselves about the periphery.

**19.** Further stage of the same process.

**20.** Merozoites are formed ready to be set free in the blood.

**21.** Adult microgametocyte.

**22.** Adult macrogamete.

**23.** Microgametocyte giving off a microgamete.

### *Figure B.*—Tertian Parasites

**24** to **30.** Small merozoites soon after entry into a red cell showing the varying morphology of the chromatin and the ring form of the parasite. Fig. 28 shows the peculiar malarial dotting which is characteristic of the red cell infected by the tertian parasite.

**31** and **32.** Parasites about eight hours old beginning to throw out pseudopodia.

**33, 34,** and **35.** Amœboid forms under the action of quinine.

**36** and **37.** Beginning formation of pigment.

**38, 39, 40, 41, 42,** and **43.** Parasites 24 to 36 hours old.

**44.** Triple infection of red cell.

**45.** Macrogamete.

**46.** Microgametocyte.

**47, 48, 49, 50, 51,** and **52.** Various stages of development of the segmenting parasite.

**53.** Early stage of segmentation.

**54.** Slightly more advanced.

**55.** Red cell ruptured and parasite set free.

**56.** Microgametocyte giving off microgamete.

### *Figure C.*—Æstivo-autumnal Parasites

**57, 58, 59,** and **60.** Small ring forms showing the spherical masses of chromatin and the fine blue bow of the ring.

**61.** Blood cell showing granular degeneration and containing two parasites.

**62.** Twenty-four-hour-old parasite containing pigment.

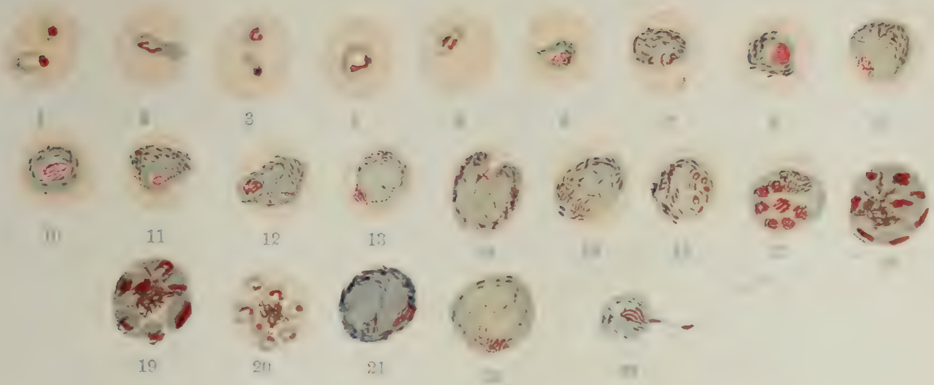
**63, 64, 65,** and **66.** Various stages of growth toward segmentation.

**67** and **68.** Microgametocytes.

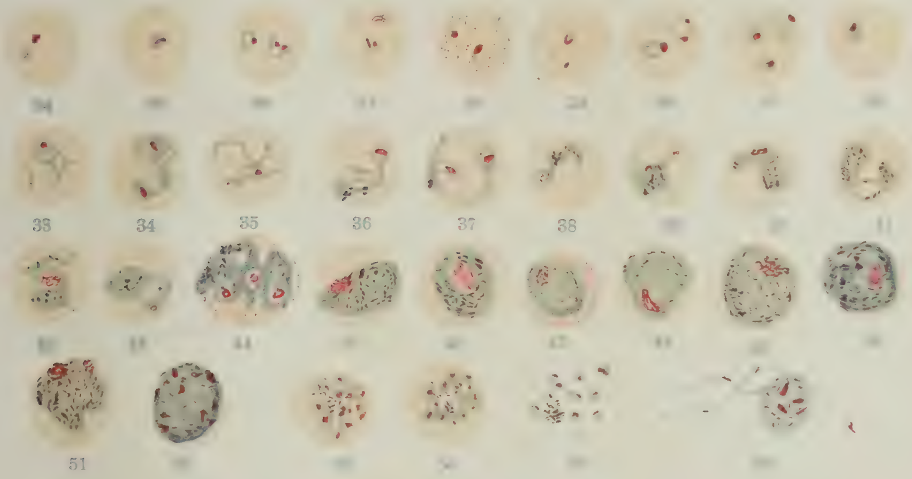
**69, 70,** and **71.** Macrogametes.

**72.** Microgametocyte after the blood is shed. The crescent form is changed into the so-called spherical body.

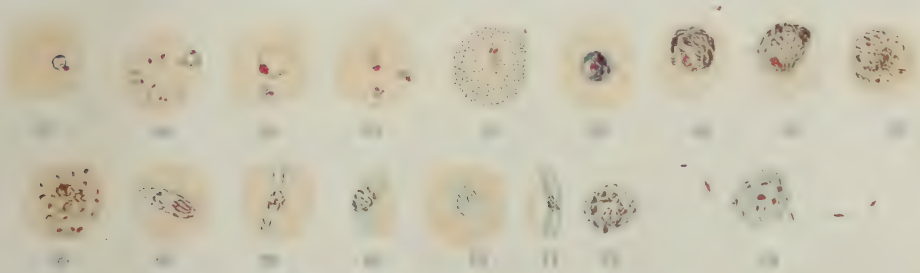
**73.** Microgametocyte giving off microgamete. The crescent form shown in Fig. 67 changes to the spherical form as soon as the blood is shed.



A.—QUARTAN PARASITE.



B.—TERTIAN PARASITE.



C.—ÆSTIVO-AUTUMNAL PARASITE.

PLATE IX.—MALARIAL PARASITES.

segmentation, in fresh blood and stained smears from a case of tertian malaria. The parasites attach themselves to the edge of the red cell and flatten out along the rims, sending into the cell substance a number of small pseudopodial projections. These become larger, and the substance of the rim flows over the parasite until the latter is wholly enclosed within the border of the cell. As the parasite enters the cell the pseudopodia extending from the two ends come together and produce the familiar ring form, usually enclosing a small portion of the red cell during this process. This enclosure soon becomes decolorized and absorbed.

The process thus recorded differs somewhat, however, from that observed by Schaudinn,[1] who noted that the parasite entered the red cell in a forcible manner similar to that observed in the penetration of the vermicules into the cells of the mosquito's stomach.

**The sexual cycle** is carried out by the crescents which are specialized forms of cells. One of these, the microgametocyte, has the power to form spermatozoa, and by them to fertilize the female organism or macrogamete.

These crescents are formed chiefly in the bone marrow from small ovoid intracellular bodies, which are early in their development distinguished from the ordinary amœboid forms by their more abundant and coarse pigment and circular outline and centrally placed nucleus.[2] They do not undergo segmentation while in the blood. The adult crescents are quite constantly present in well-developed cases, and are often found in the blood after treatment with quinine has caused the disappearance of the amœboid bodies, the power of resisting the action of drugs being much more marked in the crescents than in any other form of the plasmodium. There is a certain amount of evidence to show that the crescents may under special conditions give rise to a fresh group of merozoites and thus incite a recrudescence of the disease even after all the amœboid forms have been destroyed by quinine.

Two types of crescents may be distinguished morphologically, both of which begin as small amœboid forms and gradually mature into oval or crescentic forms. One of these, the microgametocyte (Plate IX, Fig. 67), or the cell producing the male elements, has the power of becoming flagellated and giving off the flagellum (microgamete); the other, the macrogametes (female elements), do not give off flagella. The microgametocytes are distinguished from the macrogametes by the fact that in the former (the male form) the pigment is usually scattered throughout the body of the crescent, the chromatin is more abundant, is in the form of a loose network, and the entire body stains rather faintly; while in the female form the pigment surrounds the rather scanty compact nuclear chromatin in a ring form, the cell body stains deeply, is more crescentic in shape and longer and narrower than the male, and no flagella are given off (Plate IX, Figs. 68, 69, 71). The crescents in the fresh blood show no amœboid motion, and even the pigment is motionless. They are either crescentic in form with the pigment collected at the center, or they may be spindle-shaped with somewhat scattered pigment, or, finally, short, thick, or sausage-shaped bodies with pigment irregularly scattered, or, more frequently, gathered into a ring about the nucleus. They are all endoglobular, the faint remnant of the red cell often being seen as a faint line stretching between the two horns of the crescent (Plate IX, Figs. 69, 71). The formation of flagella (microgametes) does not take place in the circulating blood; it begins only after the blood has been on a slide for fifteen or thirty minutes or has remained for some time in the stomach of a mosquito. Both male and female crescents after the blood is shed assume an oval or spherical form (Plate IX, Fig. 72), and the pigment becomes motile. The flagella are given off from the male form.

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[1] Arb. a. d. Kais. Gesundheitsamte, 1902, vol. xix, p. 169.

[2] *Stephens* and *Christophers:* Rep. to the Malaria Com. of the Royal Society, London, Series iii, p. 8.

These flagella, usually about four in number (Plate IX, Fig. 73), bud out from the periphery of one of the microgametocytes, which has previously assumed a spherical instead of a crescent shape and become actively amœboid. The flagella grow to a length of three to five times the diameter of the red cell. They are either pointed or bulbous at their extremities, or they present swellings at irregular intervals along their extent. Their motion is rather rapid in warm stage preparations, and they finally become detached and move about free in the serum. The pigment during this process usually remains at the center of the spherical mass, and is actively motile; but in preparations stained to show the nuclear chromatin, the latter may be seen to penetrate the flagella in the form of long thin rods, which remain after the flagella become detached. The shell of the red cell in which the crescent developed can rarely be seen in fresh preparations of the flagellate forms, as the organism is usually set free before flagellation. One of the macrogametes, in which reduction of the chromatin by extrusion of a polar body has occurred, is penetrated by a flagellum and fertilized. This process, which seems necessary for the continuance of the race, is normally carried out in the middle intestine of the mosquito of the genus Anopheles, no other species as yet having been found capable of acting as an extra-human host.

The actual entry of the microgamete (spermatozoon) into the macrogamete was first observed by MacCallum[1] in a fresh blood preparation in a case of Halteridium infection in the blood of a crow. If a patient in whose blood are present mature crescents is bitten by an Anopheles and the atmospheric temperature is sufficiently high for the growth of the organism in the mosquito, fertilization takes place and the oökinet or vermicule so formed in the lumen of the intestine penetrates the wall of that viscus, and at the end of two days the fertilized cysts may be found adherent to the wall of the intestine in the form of small, pigmented, oval bodies, 8 to 10 micra in diameter containing the pigment of the original gametes.

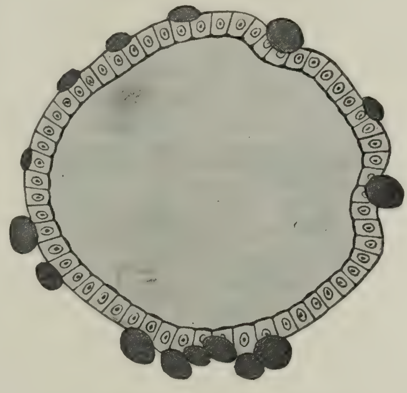
Fig. 47.—Diagram of Cross-section of Stomach of Mosquito, Showing Zygotes or Oöcysts Containing Sporozoites.

Two days later these zygotes or oöcysts are much larger and have a distinct capsule, while by the sixth day they may measure 60 to 80 micra in diameter (Fig. 48). They contain numerous small bodies, which are nuclei due to the frequent division of the original nuclear material, and the capsule is much thicker. Each one of the bodies or sporoblasts so formed is a spherical cell which

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[1] Journal of Exp. Medicine, 1898, vol. iii, p. 117.

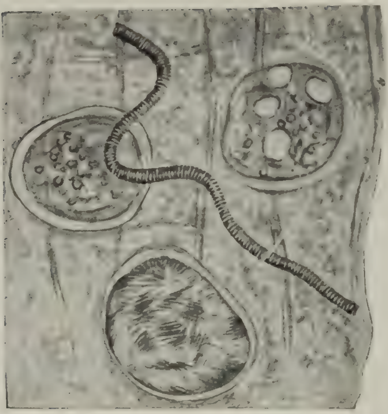

Fig. 48.—Fresh Preparation of Wall of Stomach of Infected Anopheles, Showing Oöcysts in Various Stages of Development. (Nuttall.)

at the end of a week contains a large number of slender, thread-like rods, twelve to sixteen micra in length, with pointed extremities, each one of these rods having a small mass of nuclear chromatin (Fig. 49). The cyst projects through the wall of the intestine into the cœlom cavity of the mosquito host, and when it ruptures, the minute rods or sporozoites with which it is filled are carried by the lymph currents to the salivary glands, from which they may be injected with the saliva when the female Anopheles bites another subject.

The sporozoites, after entering the circulation in man, attack the red cells and become the small amœboid forms described above. The actual entry of the sporozoite has naturally not been observed in the circulating blood, but Schaudinn[1] describes the phenomenon as observed *in vitro*, using human blood and fresh sporozoites obtained by dissecting an infected mosquito. In two to three weeks the formation of the gametes takes place and the crescent forms appear in the blood.

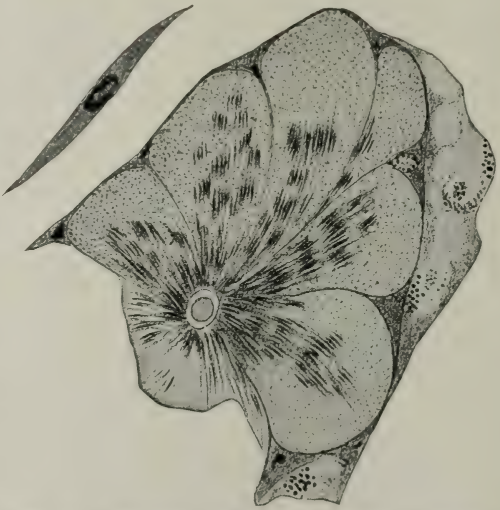

Fig. 49.—Section of Middle Lobe of Salivary Gland of Anopheles, Showing Sporozoites. A more highly magnified sporozoite is shown at the side. (Grassi.)

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[1] Arb. aus d. Kais. Gesundheitsamte, 1902, Bd. xix, p. 169.

### MODE OF INFECTION

That this is possible has been abundantly proven by allowing mosquitoes containing æstivo-autumnal sporozoites to bite healthy persons, who, after a period of incubation of about ten days, were seized with an æstivo-autumnal type of fever, and the characteristic organisms were found in the blood, though not present previously.

### DEVELOPMENT OF THE TERTIAN PARASITE

The earliest form of tertian parasite is about one-fifth of the diameter of the red cell which contains it, and in fresh blood resembles nothing so much as one of the vacuoles often present in the bodies of the red cells, except that it possesses active amœboid motion in the substance of the corpuscle (Plate IX, Figs. 24, 32). When stained in a dried smear the parasite takes the form of a small ring, with a central clear space in which lies the minute spherical nucleus, which can be often stained by thionin or an old Jenner staining mixture, but more certainly by the combination of stains devised by Nocht and modified by Giemsa and others. There is no pigment in the parasite in the early stages and little or no change in the red cell.

During the eight hours succeeding the entrance of the merozoite into the body of the red cell the growth is rapid and the organism assumes the form of a ring with a large mass on one side, the so-called signet form. The nucleus changes from a solid, deeply staining mass to a collection of fine nuclear particles very close together, much more compact and deeply staining, however, than in the mature forms. The signet portion of the body of the parasite gives out long pseudopodia (Figs. 33–37).

The ring at this stage is to be distinguished from the æstivo-autumnal ring by the fact that it is coarser in outline and contains pigment, while the æstivo-autumnal is very delicate in form and at this stage contains no pigment. The red cell can now be seen to have swollen and become paler, and in stained preparations many of the erythrocytes show the fine dotting of the protoplasm first noted by Mannaberg[1] and described in detail by Schüffner[2] (Plate IX, Figs. 28, 44, and 47).

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[1] Die Malaria-Krankheiten. Wien, 1899.

[2] Deut. Arch. f. klin. Med., 1899, Bd. lxiv, p. 428.

During the next twenty-four hours the parasite grows to a large irregularly shaped amœboid mass which fills about three-fourths of the red cell and contains much fine pigment and a nucleus which is composed of fine granules staining rather feebly (Plate IX, Figs. 38–44). The nucleus is much fainter than in the ring forms and lies in a clear area at one side of the parasite. Little change is noted in the ensuing twenty-four hours, except a gradual increase in the size of the parasite and a diminution of the hæmoglobin content of the invaded red cell, until about eight hours before the chill. At this time the homogeneous staining of the body of the parasite changes and shows a reticular structure. The pigment gradually collects at the center of the parasite and the white spots in the reticulum become more marked, while a nuclear stain shows that each one of these areas contains a compact, deeply staining nucleus similar to that seen in the early form of the parasite after it has invaded the red cell. Occasionally one-half of the schizont undergoes these changes while the other half remains unchanged. The segmentation is only irregular in time in these forms and is completed later. The number of merozoites formed is from fifteen to twenty, though smaller numbers are seen (Figs. 52–55).

A number of the large forms do not segment. These are the gametes, and if observed on a warm stage will give out flagella if male, or receive them if female. Accurate morphological differences have not been established with desirable clearness between the male and the female gametes, which will always enable a distinction to be made before the special sexual process takes place; but in general it may be said that the macrogamete (Figs. 45 and 50) stains deeply, the chromatin is compact and laterally placed, the achromatic zone is narrow and the pigment black and scattered over the whole parasite. The male form, on the other hand, stains relatively faintly, the chromatin is in coarse threads and centrally placed, or extends in a band across the cell; the achromatic zone is large, and the pigment is coarse and chiefly peripheral. (See Plate IX, Figs. 46, 47, 48, 49.)

The merozoites formed by the breaking up of the segmenting forms and set free in the blood by the rupture of the red cell, immediately enter other red cells and continue the asexual cycle, which occupies for its completion forty-eight hours.

### DEVELOPMENT OF THE QUARTAN PARASITE

The early forms of the quartan parasite can not always be distinguished from those of the tertian until after the formation of pigment, when it will be seen that the quartan pigment is in much larger blocks than that produced by the tertian (Plate IX, Figs. 1–7). In fresh preparations, the pigment together with the higher refractive power, the slow amœboid motions of the organism, and the lack of pseudopodia, together with the shrunken appearance of the red cell, will distinguish the quartan parasite.

The mature forms, and especially those which are segmenting, are easily differentiated from the tertian (Figs. 7–20). The reticulum is coarser and the number of merozoites formed smaller than in the tertian, the average being six to twelve, each with a well-marked nucleus (Figs. 16–20). The pigment arranges itself along the line of division between the merozoites and does not collect in a single mass at the center of the rosette, as in the tertian. The rosette itself is much more regular in form than with the tertian, which is often merely an irregular mass, while the quartan forms the so-called "daisy" shape. As the time required for development is seventy-two hours, and the entire process takes place in the peripheral blood, the number of segmenting parasites in different stages in the blood of a patient suffering from quartan fever is often very large. The gametes of the quartan parasite (Plate IX, Figs. 21 and 22) are distinguished from the amœboid forms by the same differences in morphology as have been described for the tertian.

If the blood of a person suffering from either tertian or quartan fever is taken into the intestine of an Anopheles, the same process of fertilization and the formation of oöcysts is carried out as has been described in the case of the æstivo-autumnal type, with only slight variations in the morphology of the various stages.

### CHANGES IN THE RED AND WHITE CELLS IN MALARIA

Evidences of degeneration in the red cells are quite common. The quartan parasite usually causes the red cell to shrink and become slightly crenated, but this crenation can often be observed in the red cell infected with tertian organisms, especially in smears. In a few of these cells there are basophile granules (Plate IX, Fig.

61) which stain well with either thionin or methylene blue, but these basophile granules are much more abundant in the cells which contain no organisms.

In the infected and shriveled cells containing tertian organisms, small granules are often to be seen in preparations with a chromatin stain, which are smaller and more abundant than the basophile granules (Plate IX, Figs. 28, 44, 47). These small reddish granules are considered by many observers as an early stage of granular degeneration. Maurer[1] suggests that they are degenerated particles of the stroma of the red cell. Others have considered these fine dots as remnants of the hæmoglobin of the cell which collect in masses as the cell swells and loses its coloring matter. In the other types of malaria in which the red cells shrink and become deeper in color no such fine granulation has been observed, though, as stated on page 199, coarse dots may be seen in æstival infection. Mannaberg suggests that they may be caused by coagulation of the substance of the red cell by the poison of the parasite.

The degree of destruction of the red cells in malaria is dependent upon the number of parasites present. It may reach a very extraordinary figure, cases having been reported where the red cells were reduced to 500,000, while those remaining showed the changes of an anæmia of a pernicious type. As a rule, the reduction is rapid at first, but becomes less marked as the disease progresses, especially when the cells become reduced to 2,000,000.

The leucocytes are not, as a rule, much altered. A moderate leucocytosis is present during the chill, with a relative increase in the large mononuclear forms, up to 20 or 25 per cent., while during apyrexia there is a fairly marked leucopenia.[2] In chronic cases there is a good deal of pigment in the bodies of the leucocytes, the large mononuclears chiefly, but occasionally in the small lymphocytes and polynuclears.

Multiple infection of the same red cell by two or more organisms is frequently seen in the æstivo-autumnal fevers (Plate IX, Fig. 58), and occasionally in the tertians with numerous parasites (Figs. 31, 44), but only rarely in quartan fevers. The condition usually observed is the infection of a red cell with two or more rings, five and six have been seen in æstivo-autumnal cases. In the tertian the double infection is the most frequent. Usually the rings are separate, but rarely they may overlie each other or their pseudopodia may intertwine to form the most bizarre figures, especially after the administration of quinine. Double infection with mature organisms is rarely seen. (See Plate IX, Fig. 44.)

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[1] Cent. f. Bakt., 1900, Bd. xxviii, p. 114. *Schüffner:* Deut. Arch. f. klin. Med., 1899, Bd. lxiv, p. 428.

[2] *Billet:* Thirteenth Internat. Cong. of Medicine, 1900. *Pöch:* Zeit. f. Hygiene, 1903, Bd. xlii, p. 563.

Mixed infection with two different species of organisms is not uncommon, especially in the tropics. The tertian and the æstivo-autumnal form the most frequent combination. A mixed infection of malaria and typhoid fever is rarely seen. When it exists the plasmodia are generally absent from the blood during the active course of the typhoid and only reappear with the lowering of the fever and convalescence.

## METHODS OF EXAMINING BLOOD FOR MALARIAL PARASITES

It is not necessary to obtain blood for the purpose of making a diagnosis of malaria at any particular time in relation to the chill, because if the patient has the disease the parasites will always be found if sufficient care is taken in searching for them, only excepting in the blood of persons to whom quinine has been administered, and in some cases of black-water fever where the parasites may not appear in the peripheral blood. Even half a gram of the quinine is sufficient to nullify a most careful search for the plasmodia, so that a negative result under these circumstances is of little value.

The examination of fresh preparations is the simplest means for a mere diagnosis of tertian or quartan malaria if the blood can be examined at the bedside, using the technique which has been given under the general discussion of the methods of examination of the blood. If the blood can not be examined immediately it is far better to make a number of large smears on slides, allow them to dry, fix and stain them with thionin, or better, with eosin and methylene azure, and search through the preparations at leisure with a mechanical stage. This is especially advisable in those cases in which quinine has previously been administered, and in cases in which the blood is taken some hours after the chill in tertian and quartan malaria with a single group of organisms.

The small ring forms may be very scanty, and their recognition in the fresh preparation quite impossible, while in a well-stained slide the recognition of the rings is a simple matter even to the beginner. In searching for æstival parasites, fresh preparations should not be relied upon, for while it is often possible to find crescentic forms and thus make a diagnosis, when the latter are scanty the very small ring forms are often overlooked. The writer has seen a number of mistakes of this sort which would have been avoided if a properly stained eosin-azure preparation had been employed. If the parasite can not be found by ordinary means, a very thick smear should be made and the hæmoglobin removed by placing the slide, without fixing, in a weak solution of watery eosin, and then staining with dilute methylene azure. Ruge[1] has suggested a modification of this method which we owe to Ross. Thick smears are fixed in a fluid containing 2 per cent. formalin and 1 per cent. acetic acid. The slide may now be stained by methylene blue or thionin without any risk of dissolving the cells.

The diagnosis of malaria from pigment in the leucocytes is extremely hazardous, nor is the increase in large mononuclears of much greater importance, though both of these points may be of some use in the diagnosis of malaria after large doses of quinine have been administered.

Stephens and Christophers[2] regard 10 per cent. of large mononuclears as suggestive and 15 to 20 per cent. as practically diagnostic of malaria. Malarial pigment in the blood is almost always in these cells, and usually in large quantities; particles outside of the cells may be assumed to be dirt derived from the slide or the skin of the finger.

Flagellation, fertilization, and other vital phenomena are studied best in fresh preparations and on a warm stage, then the details observed in stained smears made from the same slide. A useful means of studying flagellation has been suggested by the Italian observers. They make rather thick smears and keep them in a moist chamber for fifteen to thirty minutes and then dry and stain by the eosin-methylene azure method.

It is important for the beginner to make a careful study of a series of slides taken about twelve hours apart from a case of tertian malaria with a single group of parasites, thus familiarizing himself with the various stages of development of the organism and also with the gametes. It will not be difficult then to recognize double infections and to prognosticate roughly from the morphology of the parasites the time of the next chill, a matter of the utmost importance in the treatment of the disease. The following table may be of use in differentiating the various types of organisms in fresh blood:

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[1] Deut. med. Woch., 1903, vol. xxix, p. 205.

[2] Loc. cit., p. 41.

| | QUARTAN. | TERTIAN. | ÆSTIVO-AUTUMNAL. |
|---|---|---|---|
| 1. Size. | Small. | Large. | Small forms only in the peripheral blood. |
| 2. Contour. | Sharp. | More hazy. | Sharp. |
| 3. Refraction. | Highly refractile. | Less so. | Highly refractile. |
| 4. Activity. | Motion slow. | Motion very active. | Very active. |
| 5. Pigment. | Coarse and near periphery. | Fine and scattered throughout. | Fine and less abundant. |
| 6. Segmenting forms. | 6–12 segments, nuclei well marked. | 15–20 segments, nuclei not easily seen. | Variable number, usually 7 to 15. |
| 7. Red cells. | Dark in color. | Swollen, pale, and dotted. | Often shrink and become of a brassy color. |
| 8. Cycle. | 72 hours. | 48 hours. | 24 to 48 hours. |
| 9. Gametes. | Small. | Large. | In crescentic or oval form. |
| 10. Site of segmentation. | Chiefly in the peripheral blood. | In the peripheral blood, but also extensively in the organs. | Almost entirely in spleen, bone marrow, and capillary vessels of the brain and other viscera. |

### THE RELATION OF THE PARASITES TO THE TEMPERATURE CURVE

The mere inspection of the temperature chart is not in itself sufficient to determine the cycle of development of the parasite, for it is well known that the daily chill may be produced by any one of three forms of organisms. The æstivo-autumnal, for instance, may cause a daily chill by a group of parasites segmenting every twenty-four hours; the tertian may also cause a quotidian fever by a double infection, the two groups of parasites segmenting on alternate days; while the quartan may produce the same phenomenon by a triple infection, each group segmenting twenty-four hours apart.

Inasmuch as the rise in temperature corresponds very closely to the setting free of the parasites in the blood we would expect on examining a double tertian infection to find both the small ring forms just liberated by the segmentation, and also mature pigmented parasites twenty-four hours old. At a period twelve hours before or after a chill the only parasites would be small amœboid forms with some pigment, and large parasites in the presegmenting stage plus male and female gametes.

To determine the cycle of the parasite it is necessary to make examinations at intervals of about four hours, the temperature being plotted at the same time. The number of parasites of the same size at each observation should be determined by a thorough search through one or more smears, the number depending upon the severity of the infection, and a curve drawn showing the gradual formation of the different groups. The tracing of the double tertian and triple quartan groups is not a matter of great difficulty, but in the æstivo-autumnal fevers the time of segmentation is much more irregular than in the two preceding forms, and as the temperature is often high and continuous and the segmenting parasites do not appear in the blood, the exact time required for development is still somewhat in doubt. There are some observers, however, who deny the twenty-four hours' period of segmentation for this parasite and think that one generation segmenting at intervals of forty-eight hours can produce a daily fever. It is also possible that the segmentation takes place in the spleen at very irregular intervals, thus producing a series of crops of small parasites which may not produce a marked rise in temperature unless a sufficient number of them be present in the circulating blood.

## THE ACTION OF QUININE

Besides the well-known action of quinine in curing malaria, the morphological changes which it produces are of interest.

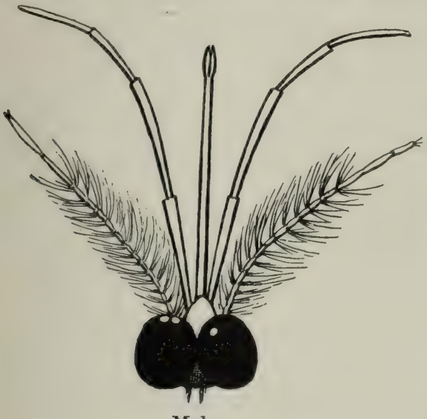
A large dose of quinine does not necessarily prevent segmentation of large forms, but it may destroy the small merozoites as soon as they are set free in the blood, and both alter the morphology and prevent the development of the ring forms in the red cells. The rings do not stain so deeply as normal; their edges are without sharp contour, and the whole parasite appears as if torn. The nucleus is altered in shape and shrunken.

The larger amœboid forms are much altered by the action of quinine, showing very numerous pseudopodia, scattering of the chromatin in irregular masses often outside the cell, and altered staining qualities of the protoplasm of the cell body (Plate IX, Fig. 35). The younger gametes of both sexes show the same scattering of the chromatin and torn appearance of the protoplasm as in the amœboid cycle, but the adult sexual parasites show no change.[1]

In black-water fever, which is generally regarded as a severe infection of the æstivo-autumnal type with but very few parasites in the peripheral blood, the administration of quinine is apt to cause a marked hæmoglobinuria within a short time.

### IDENTIFICATION AND STUDY OF MOSQUITOES

The identification of the form of mosquito which acts as host for the parasite of human malaria, and the demonstration of the sporoblasts in the wall of the midgut, while not belonging strictly to clinical diagnosis may be briefly outlined, as the knowledge so obtained is often of importance in the tracing of local epidemics and in planning measures for the destruction of the infecting insects.



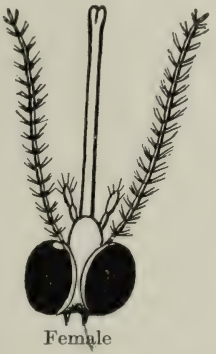


Fig. 50.—Proboscis and Palpi of Culex. (Braun.)

As stated in a previous paragraph, the various members of the genus Anophelina are responsible for the development and reinoculation into man of the parasites of human malaria. The male and female of this genus may be distinguished with some ease from the members of the genus Culex by observing the relation between the length of the proboscis and the palpi. As is shown in Figs. 50 and 51, the palpi in the female of the Culex group are relatively very short, while those in the female of the Anopheles group are equal in length to the proboscis. These facts may be made out by the use of a hand lens and are sufficient for the preliminary classification into genera, but the exact determination of the species requires careful study of minute anatomical details and need not be considered here.

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[1] See plates in *Schaudinn:* Arb. aus d. Kais. Gesundheitsamte, 1902, Plate V, Figs. 81 to 89.

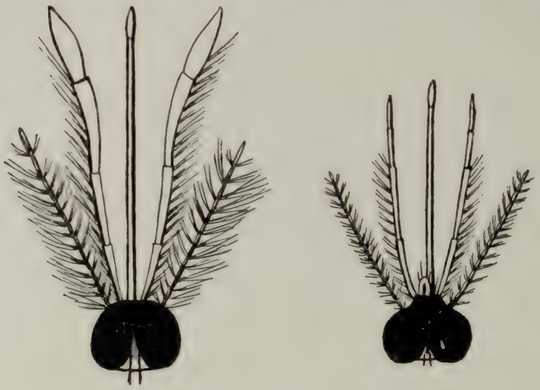
Male Female

Fig. 51.—Proboscis and Palpi of Anopheles. (Braun.)

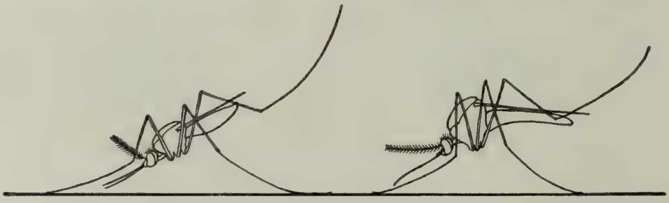
*a* *b*

Fig. 52.—Attitude Assumed by (*a*) Anopheles and (*b*) Culex when Resting.

In examining dwellings for specimens of Anopheles it is well to remember that they are strictly nocturnal in their habits and are most likely to be found in the daytime on the walls or ceilings of dark rooms. The attitude assumed by Anopheles when resting is somewhat characteristic and may aid in identification. (See Fig. 52.)

In order to demonstrate malarial infection in the mosquito it is necessary to dissect the insect so that the midgut to which the sporoblasts are attached may be examined with a high power. To do this, the freshly caught mosquito is killed with chloroform, tobacco smoke, or the vapor from potassium cyanide, and the wings and legs pulled off with forceps. The remainder of the insect is then placed on a slide and covered with a few drops of physiological saline solution. The posterior portion of the abdomen is then gently flattened with a needle and two nicks are made, one on each side of the abdomen between the third and the last two segments. The thorax is then transfixed with another needle and gentle traction is made on the last two segments of the abdomen. As the two portions of the exoskeleton are dragged apart a bundle of white tubes make their appearance. These are the intestine and the Malpighian tubes, while on further traction the midgut and the œsophagus make their appearance.

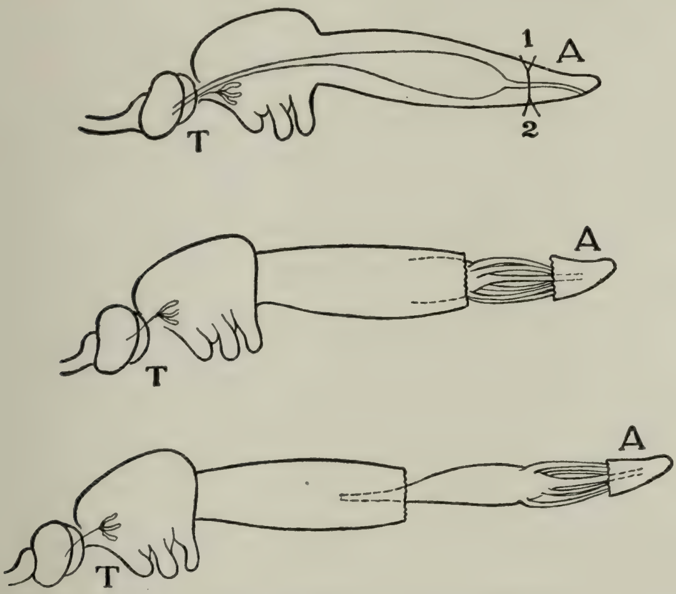

Fig. 53.—Diagram Showing the Stages of the Dissection of the Stomach of the Mosquito.

The needle should now be transferred from the terminal segments of the thorax to the upper portion of the intestine to avoid tearing that viscus. As soon as the intestine and its appendages are separated from the thorax they should be separated by cutting with a needle just below the saccular termination of the midgut, and the latter isolated. Fresh salt solution is then added and the gut covered with a cover glass and examined with a one-twelfth oil immersion lens. The smaller zygotes may be recognized by the pigment which they contain, derived from the fertilized macrogamete. They measure some 10 micra in diameter and must be carefully distinguished from the large swollen epithelial cells of the stomach wall which do not contain pigment. The larger cysts are easily identified by the sporozoite rods with which they are now filled. The latter may be set free in the fluid by pressing on the cover until the cysts rupture. Permanent preparations may be made by drying the slide and staining with eosin-azure after a few minutes' fixation in alcohol.

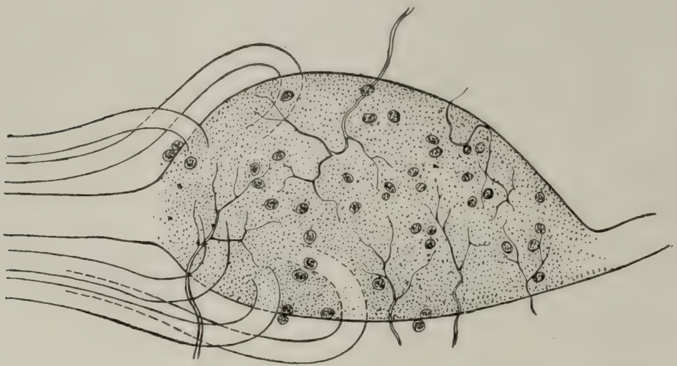

FIG. 54.—LATERAL VIEW OF STOMACH OF MOSQUITO, SHOWING ZYGOTES OR CYSTS CONTAINING SPOROZOITES. (Daniels.)

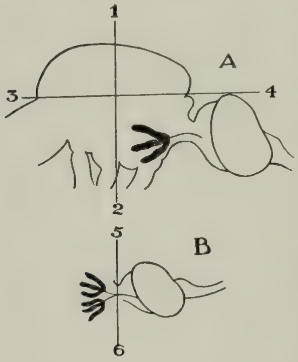

FIG. 55.—DIAGRAM OF DISSECTION OF SALIVARY GLANDS OF MOSQUITO.

An incision should be made along the line 3–4, then a second along 1–2, and the head and thorax dragged apart. (Daniels.)

The salivary glands may be obtained by fixation of the thorax with a needle and then dragging on the head of the mosquito. If the preparation is then examined with a half-inch lens the glands may be seen as glistening transparent bodies attached to the neck. They should be separated from the head by a needle cut and examined in salt solution. The sporozoites are most abundant in the middle lobe and may be set free by gentle pressure on the cover glass.

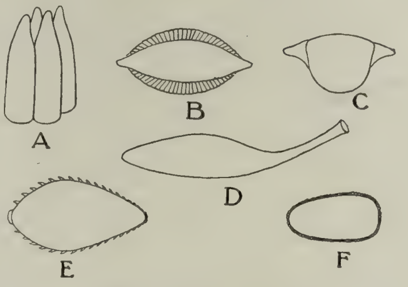

FIG. 56.—*a*, Eggs of Culex; *b* and *c*, eggs of Anopheles; *d*, egg of Panoplites; *e*, egg of Psorophora; *f*, egg of Stegomyia. (Daniels.)

The eggs of mosquitoes of different genera vary greatly in their morphology. In most cases they are laid on the surface of water. The identification of the eggs is occasionally of interest in determining the species, which may be of importance in the spread of either malaria or yellow fever. Fig. 56 gives sufficient details for this purpose.[1]

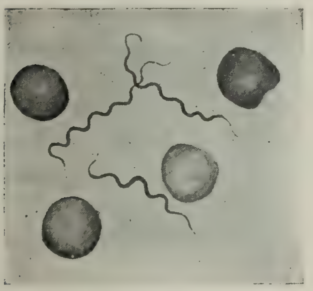

FIG. 57.—SPIROCHÆTE OF OBERMEIER.

## RELAPSING FEVER

During the paroxysms of relapsing fever a parasite has been found [2] in the peripheral circulation which in its general morphology corresponds to the spirillum type of bacteria. The organism measures about 30 to 40 micra in length. Between the attacks of fever the parasites are not found in the blood.

Manson [3] has described a case of recurrent fever in which small spirilla were demonstrated which differed somewhat in morphology from the Spirochæte of Obermeier. The body was somewhat more slender and the curves were less sharp than are commonly seen in this parasite.

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[1] Further details on the identification and the minute anatomy of mosquitoes may be found in:—*Stephens* and *Christophers:* Practical Study of Malaria, London, 1903. *Daniels:* Laboratory Studies in Tropical Medicine, London, 1903. *Nuttall* and *Shipley:* Journ. of Hygiene, vols. i and iii. *Theobald:* Monograph of the Culicidæ, London. *Berkeley:* Laboratory Work with Mosquitoes, New York, 1902.

[2] *Obermeier:* Cent. f. d. med. Wissen., 1873.

[3] British Med. Journal, 1903, p. 538.

A spiral with very wide turns has also been found in persons suffering from African relapsing or tick fever, and has been named Spirochæte Duttoni. Relapsing fever occurs but rarely in the United States, and is chiefly seen in Russia, India, and Africa.[1]

## FILARIASIS

**Filaria nocturna.**—In the disease known as filariasis the inciting agent is a nematode worm, Filaria Bancrofti. The adult male and female organisms of this species live in the lymphatics of the abdomen and pelvis in man. The female Filaria Bancrofti gives off an enormous number of ova which develop into small motile embryos and circulate in the blood. These embryos are called Filaria nocturna.

The presence of the worm and its embryos may, as is usually the case, give rise to no symptoms, or may cause chyluria and hæmaturia or the enlargement of a few lymph vessels or lymph nodes in the groin, or the swelling and thickening of an entire limb or portion of the body, the latter condition being known as elephantiasis. In the United States,Central America, and the West Indies, most of the cases either give no symptoms or show merely a moderate chyluria and hæmaturia or a few dilated lymph channels. In the countries above mentioned advanced elephantiasis is uncommon, but in the islands of the Pacific it is extremely frequent.

Most of the cases of filariasis observed in the United States are imported from the islands of the West Indies or from Central America, but infections with this parasite have been observed in persons who have never left the northern part of the United States. In the Southern States the disease is fairly frequent. The infection in some portions of China and India involves one-third of the population; in Samoa and the Fiji Islands about 50 per cent. of the inhabitants are affected, judging from the elephantiasis alone. Probably more than this per cent. have parasites in the blood. A large proportion of the natives of some of the islands of the West Indies[1] and of the Isthmus of Panama are said to be infected.

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[1] A valuable monograph is that of *Laveran* and *Mesnil,* trans. by *Nabarro,* Trypanosomes and Trypanosomiases, 1907. For studies on Spirochæte Duttoni see *Novy* and *Knapp:* Jour. of Infectious Diseases, vol. iii, 1906, p. 291; *Breinl* and *Kinghorn:* Memoir XXI of The Liverpool School of Tropical Medicine, 1906, p. 1. Other papers of interest are *Schaudinn:* Arb. a. d. kais. Gesundheitsamte, Bd. xx, 1904, p. 387; *Lancaster:* Quarterly Review, 1904, p. 113.

The male and female adult worms live in the thoracic duct, in the lymph vessels of the scrotum, and in the lymph nodes especially those in the groin. There may be but a single pair of these worms or a large number of pairs which often are entangled in an inextricable mass. They are long, white, transparent worms measuring from eight to ten centimeters in length and about a millimeter in diameter. The male is slightly smaller than the female. In the female two uterine tubes occupy the greater portion of the body and are usually filled with ova in various stages

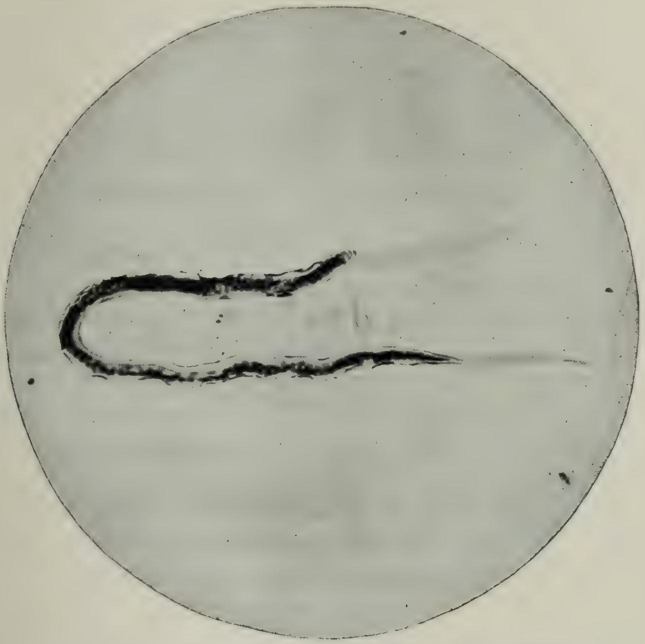

FIG. 58.—EMBRYO OF FILARIA BANCROFTI. Magnified 250 diameters. The figure shows the hyaline capsule extending beyond each end of the embryo.

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[1] *Ashford* (Filariasis in Porto Rico, Medical Record, 1903, p. 724) reports 12 per cent. of a battalion of soldiers showing filaria, and 20 per cent. of the natives having chyluria.

of development. From these the female furnishes an enormous number of embryos to the circulation, a severe infection often showing one or two to the cubic millimeter of blood. Manson estimates that in one case at least fifty million embryos must have been present in the body.

The embryos are actively motile, transparent worms, about two-tenths to four-tenths of a millimeter long and about the diameter of a red blood corpuscle, so that they can easily pass through the smallest capillaries. One end is blunt, the other tapers to a point. The embryo is sheathed in a gelatinous envelope which is considerably longer than the worm which it encloses (Fig. 58). This can be easily demonstrated by staining the parasite with eosin and methylene azure, the envelope taking a reddish stain with the eosin. The embryo itself, when stained, is seen to be filled with a large number of nuclei, and in the center a small unstained area can usually be seen which probably represents the site of the developing digestive organs of the future Filaria Bancrofti. In most of the stained specimens may be seen a clear space in the nuclei, one near the head, the other near the tail (Fig. 59). A delicate transverse striation can usually be made out when examining filariæ with high power. In fresh specimens a small prong can occasionally be noted protruding from the blunt end of the worm, usually surrounded by a sheath.

The embryos of the filaria appear in the blood only at night; during the day they occupy the vessels of the abdomen and especially those of the lungs. They are practically absent from the liver, spleen, bone marrow, and brain, in contrast to the infection of these organs with the malarial parasite. This phenomenon has been explained on the supposition that the capillaries are dilated when the patient is in bed, thus giving greater freedom for the passage of the embryos; but Manson thinks that this is not sufficient, because the parasites begin to appear about five or six o'clock in the evening before the patient has gone to bed, and an occasional one may be found during the day.

The nocturnal habits of the embryo can be reversed by causing the patient to remain in bed during the day and be up and about during the night, in which case the parasites appear in the circulation during the daytime and disappear at night.

The embryos begin to appear in the peripheral circulation in considerable numbers about eight o'clock in the evening, reach a maximum about midnight, and gradually disappear before the morning. A very few can often be demonstrated during the daytime in advanced infections with numerous parasites.

Filaria embryos and, more rarely, the adult worms, may be found in the bloody or chylous urine which is occasionally passed by patients subject to this disease, or in the fluid obtained by aspirating one of the swollen lymph nodes in the groin. The diagnosis of filariasis is more easily made, however, by searching the blood obtained from the patient during the night. If the examination can be carried out at once, very thick spreads of blood should be made and covered with a large cover glass to prevent drying, and then the slide set aside for a few minutes in order to allow the parasites to clear a space in the corpuscles by their active motion and so become visible. The preparation should be searched with a medium power lens; one of eight or sixteen millimeters focus is most suitable.

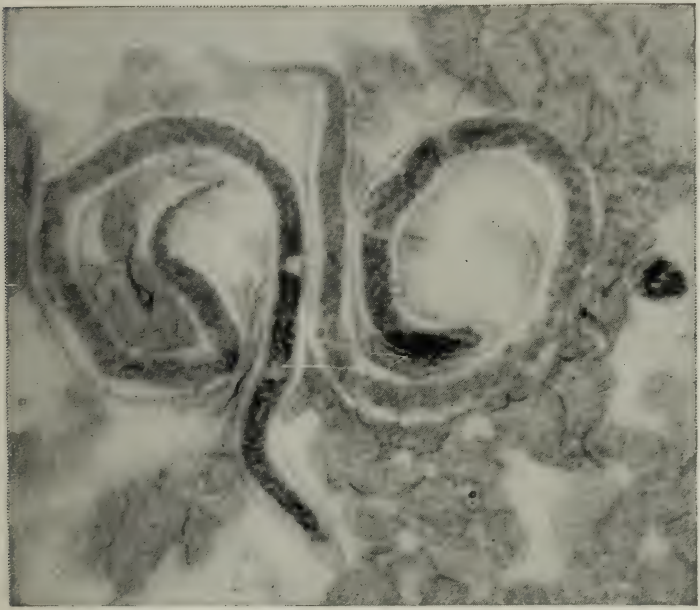

Fig. 59.—Filaria Nocturna, Showing "V Spot or Head Spot" and "Tail Spot." Magnified 900 diameters.

If the examination can not be made of the fresh preparation, thick smears should be spread and allowed to dry slowly. The slides should be fixed in either formalin-alcohol or methyl alcohol, and stained with thionin, hæmatoxylin, or eosin-methylene azure. No eosin counter-stain is needed when merely searching for the parasite; in fact, it is a distinct disadvantage.

The mode of infection in filariasis is through the mosquito as an intermediary host. The females of Culex fatigans and Anopheles nigerrimus have been shown to be capable of acting as hosts for the embryos. The embryos are drawn from the blood of the infected person into the stomach of the mosquito, and three or four hours later they will be noticed to have shed their gelatinous envelope; and if the mosquito is dissected at a still later period the worms will be found to have passed through the walls of the stomach and to have entered the thoracic muscles of the insect. In from sixteen to twenty days, depending upon the temperature of the air, the embryo develops into a worm, showing the rudiments of a mouth and alimentary canal, and increases in size to about two millimeters. The worms pass forward by the thorax and neck and enter the head and coil up close to the base of the proboscis.[1] They do not enter by the salivary duct, as is the case with the malarial parasite, but break through into the base in a way not as yet actually determined, and pass along the stylets into the puncture which the mosquito makes in the skin of the person bitten. The paired parasites thus transferred to the lymph channels develop into the adult worms, and embryos soon appear in the blood.

The embryonic filaria can but rarely be demonstrated in cases of elephantiasis arabum, though there seems to be but little doubt that they are the inciting cause of the disease. It is possible that the infection in these cases is of long standing and that the adult parasites have either died off or are encapsulated, perhaps by the blocking up of the lymphatics.

The blood in cases of filariasis shows in the early stages of the infection a moderate eosinophilia, which rises when the parasites are absent from the blood and falls when they are present. It is probable that the eosinophilia is present only in fresh infections, as low percentages are occasionally seen.[2]

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[1] *Manson:* Journal of Tropical Medicine, May, 1900, p. 254. *Low:* Brit. Med. Jour., 1900, p. 1456.

[2] *Calvert:* Johns Hopkins Hosp. Bull., 1902, p. 133.

**Filaria diurna.**—A slightly smaller species of filaria embryo has been noted in blood obtained from natives of the Congo and lower Niger regions in Africa, which is considered by Manson to be a distinct species. As the name indicates, it appears in the blood during the day. Other observers are inclined to doubt the individuality of the parasite, considering it to be merely a form of Filaria nocturna.

**Filaria Demarquaii.**—This parasite was observed by Manson in blood from natives of St. Vincent, St. Lucia, Dominica and Trinidad, West Indies. It resembles Filaria nocturna in shape, but differs in size, the average measurements being two hundred micra by five micra. It is sharp-tailed, has a long sheath, and does not show the diurnal oscillation in its appearance in the blood. It also has the power of active locomotion which is not possessed by Filaria nocturna, the latter thrashing about in the blood drop without changing its place.

**Filaria Ozzardi.**—Filaria Ozzardi has been observed in the blood obtained from the Carib Indians of British Guiana. The parasites are blunt-tailed, have no sheath, and measure between one hundred and seventy and two hundred and forty micra long by four to five micra in diameter. The adult worms measure about seven cm. in length and only about half the diameter of Filaria Bancrofti. It is somewhat doubtful whether this is actually a new species.

**Filaria perstans.**—The parasite bearing this name is very frequently encountered in the blood of the negro population of West Africa, from fifty to ninety per cent. of the inhabitants harboring the parasites. It apparently does not exist in the West Indies. It does not observe any periodicity, but is present in the blood in equal numbers day and night. The embryo measures about two-tenths of a millimeter in length and four and one-half micra in diameter, and is distinguished from the Filaria nocturna by an entire absence of sheath and by the rounded nature of both ends of the embryo. It has the power of independent locomotion in the blood.

The adult worm measures seventy to eighty millimeters in length and one-tenth of a millimeter in thickness.

Apparently the presence of the worms in the body gives rise to no clinical symptoms.

| NAME OF EMBRYO | LENGTH | GREATEST THICKNESS | SHEATH | SHAPE OF HEAD | SHAPE OF TAIL | PERIODICITY | DISTANCE OF HEAD GAP FROM HEAD | ADULT (KNOWN OR SUSPECTED) |
|---|---|---|---|---|---|---|---|---|
| Filaria nocturna | mm.<br>.317 | mm.<br>.0075 | Present | Blunt | Sharply pointed | Nocturnal, in peripheral blood | mm.<br>.052 | F. Bancrofti |
| Filaria diurna | .317 | .007 | Present | Blunt | Sharply pointed | Diurnal, in peripheral blood | — | F. Loa |
| Filaria perstans | .195 | .0045 | Absent | Blunt | Blunt, truncated | None | .03 | F. perstans |
| Filaria Demarquaii | .21 | .005 | Absent | Blunt | Sharply pointed | None | .03 | F. Demarquaii |
| Filaria Ozzardi | .21 | .005 | Absent | Blunt | Blunt, truncated | None | .03 | F. Ozzardi |

**Filaria gigas.**—A new species of filaria from Sierra Leone[1] has recently been described, the embryo of which is much larger than in the forms previously mentioned. It measures 220 to 340 by 8 to 10 micra.

The head is rounded, the tail is blunt and tapering. No sheath is present.[2]

The table on page 218, from Daniels,[3] gives the chief facts concerning the morphology of the parasites.

## TRYPANOSOMIASIS

**Trypanosoma hominis (Syn. T. Castellani, T. Gambiense).**—This parasite has been found in the blood of a number of persons suffering from a febrile disease resembling malarial fever and with the development of marked cachexia. Other symptoms are a pronounced œdema of the lower eyelids and general congestion of the skin with an itchy, erythematous eruption and a cyanosis of considerable areas, suggesting the early stage of a macular leprosy. In addition, there is marked muscular weakness, enlargement of the spleen and lymph nodes, moderate temperature, and increased frequency of the pulse and respiration.

The trypanosomata are most numerous in the blood during the febrile period and are practically absent at other times. The blood showed a moderate anæmia with a relative mononucleosis and no increase in the eosinophiles in a case examined by Manson.[4] The parasites are present in large numbers in the lymph nodes, especially the cervical group.

The parasite was discovered in human blood by Nepveu[5] but its nature was first recognized by Forde[6] and Dutton.[7] It is a small, transparent, non-pigmented, actively motile organism, about ten to twenty micra in length, provided with a flagellum continuous with the lateral aspect of one side of the body. The posterior extremity is blunt, the anterior tapering and continuous with the flagellum. The parasite is always free in the serum and is never intracorpuscular.

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[1] *Prout:* Brit. Med. Jour., 1902, vol. ii, p. 881.

[2] For a very complete review of the filariæ of man and animals with bibliography, see *Annett*, *Dutton* and *Elliott:* Thompson Yates Lab. Reports, 1901, vol. iv, part i. See also *Scheube:* Krankheiten der warmen Länder, 1905, third edition. *Manson:* Tropical Diseases, London, 1903.

[3] Lab. Studies in Tropical Medicine, London, 1903.

[4] *Manson:* p. 179.

[5] Mémoirs de la soc. de biologie, 1898, t. xv, p. 337.

[6] Journal of Tropical Medicine, Sept., 1902, p. 261.

[7] Thompson Yates Laboratory Reports, 1902; British Medical Journal, 1902, p. 881.

A small centrosome can be made out just posterior to the termination of the flagellum, while near the middle of the body is an oval nucleus (Fig. 61). These two bodies and the flagellum stain red with eosin-azure. The trypanosome multiplies by fission, the centrosome dividing first, then successively, the flagellum, nucleus, and body of the parasite. Sexual differences can be made out in the parasites.

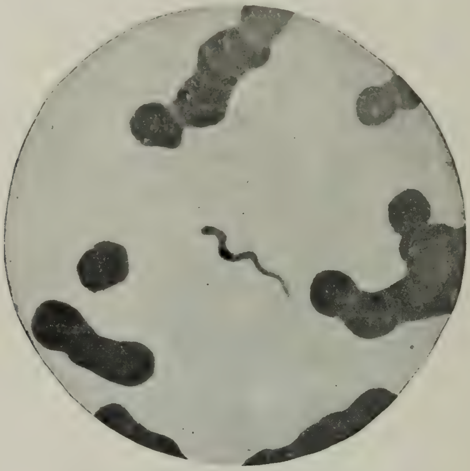

FIG. 60.—TRYPANOSOMA HOMINIS.
Photograph from a specimen in the author's possession.

Castellani[1] has found that in persons the subjects of sleeping sickness, the trypanosome could be demonstrated in the cerebrospinal fluid. It is also possible to find the same parasite in the blood, but as a rule they are few in number. Bruce[2] considers sleeping sickness a later stage of the trypanosome infection. In order to demonstrate the parasites in the spinal fluid it is necessary to centrifuge the latter and examine the deposit. The infection is probably carried by a species of tsetse fly, Glossina palpalis, which introduces the parasite into the circulation while biting.

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[1] Cent. f. Bakt., 1903, Bd. xxxv, p. 62.

[2] *Bruce, Nabarro,* and *Grieg:* Brit. Med. Journal, 1903, p. 1343.

Trypanosomata are frequently found in the blood of animals; T. Lewisi in rats; T. Evansi and Brucei in horses; and T. equiperdum in horses and donkeys. Novy and McNeal[1] have cultivated T. Lewisi and Brucei on artificial media.

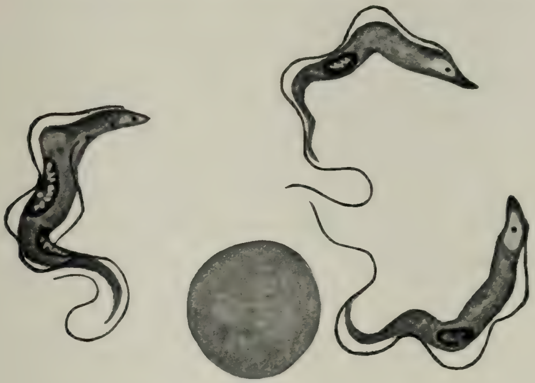

FIG. 61.—TRYPANOSOMA HOMINIS. (Dutton.)

## SPOTTED FEVER (PYROPLASMOSIS)

The disease known as spotted fever is endemic in the mountainous districts of the western portions of the United States. Investigations of the blood of persons suffering from this disease have been made and a small amœboid body has been discovered occupying the red cells.[2] In a thorough reinvestigation of the subject Stiles[3] was unable to demonstrate the parasite.

## SPIROCHÆTE PALLIDA (TREPONEMA PALLIDUM)

A minute spiral organism, Spirochæte pallida,[4] has occasionally been found in the blood of persons suffering from syphilis, and quite constantly in the primary and secondary lesions of that disease. Spirochætes have also been demonstrated, though not constantly, in tertiary lesions.

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[1] Journal of Infectious Diseases, 1904, vol. i, p. 1.

[2] *Wilson* and *Chowning:* Jour. Amer. Med. Assn., 1902, vol. cxxxix, p. 131. *Anderson:* Bull. No. 14, Hyg. Lab., U. S. Pub. Health and Mar. Hosp. Serv., Wash., 1903.

[3] Bull. No. 20, Hyg. Lab., U. S. Pub. Health and Mar. Hosp. Serv., Wash., 1904.

[4] *Schaudinn* u. *Hoffmann:* Arb. a. d. Kais. Gesundheitsamte, Bd. xxii, 1905, p. 527. *Hoffmann:* Die Aetiologie der Syphilis, Berlin, 1907.

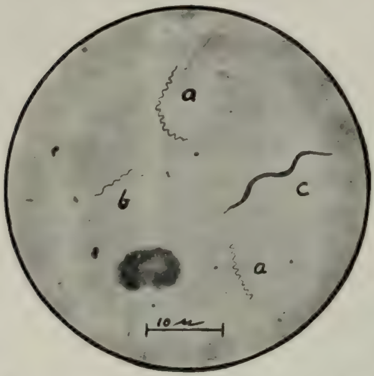

FIG. 62.—*a*, Spirochæte pallida; *b*, Spirochæte dentium; *c*, Spirochæte refringens. Redrawn from photograph and magnified 1,000 diameters. The line in the lower part of the cut is ten micra in length.

The dimensions of the organism are as follows: length, 10 to 20 micra; width, less than 0.3 micron; distance between turns, about 1.2 micra; depth of the turns, 1 to 1.5 micra; number of turns, 8 to 26. The ends are pointed.

Other spiral organisms which may be found on ulcerated surfaces are Spirochæte refringens and balanitidis; while in the mouth the Spirochæte buccalis and dentium are frequent. All except the dentium are coarser than pallida and have wide and irregular turns and rounded ends. The dentium is shorter than pallida and the turns are not so deep.

In searching for the parasite the lesion to be examined should be thoroughly cleansed; in the case of skin lesions the surface should be gently scraped with a knife. A small drop of serum is then expelled by squeezing, spread gently over a slide and allowed to dry. The specimen is fixed in strong methyl alcohol or by osmic acid (page 77), and stained with Giemsa's solution (page 86) for twenty-four hours, or for six to eight minutes with the eosin-azure mixture (page 87), and then examined with $\frac{1}{12}$ oil immersion and good illumination.

The use of some form of dark ground illuminator has been found to be of great value in the demonstration of the Spirochæte pallida in tissue juice obtained from syphilitic lesions. Successful demonstration of the parasite requires that the preparation contain no air bubbles and but few red cells. Sunlight or a strong source of artificial illumination is also needed, as well as high power, well corrected, dry lenses. The pallida shows as a spiral organism with corkscrew motion across the field.

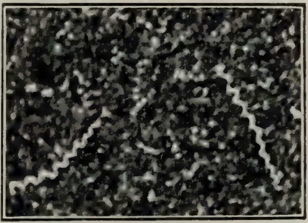

FIG. 62*a*.—SPIROCHÆTE PALLIDA. Ink method. × 1500.

A very simple means of demonstrating the Spirochæte pallida is the ink method of Burri.[1] A drop of serum obtained by squeezing the lesion or by suction with a small Bier cup is placed at one end of a glass slide and immediately mixed with a small drop of Günther Wagner India ink. The mixture is then smeared with the edge of another slide, as in making blood preparations, and should dry immediately and be of a dark brownish color. It is then examined with an oil immersion lens, the Spirochæte pallida showing as a spiral organism. Bacteria do not grow in the ink and cotton fibers and other contaminations can be easily distinguished. The only risk in diagnosis lies in confusing the Spirochæte dentium of the mouth and the Spirochæte pallida.

## VIII. BACTERIOLOGY OF THE BLOOD

The bacteriological examination of the blood[2] presupposes a working knowledge of the methods of bacteriology, and requires, if the results are to be of the slightest practical or scientific value, a great deal of time and careful work. The application of this method to the study of the blood is therefore usually beyond the means of the practitioner.

### TECHNIQUE OF BLOOD CULTURES

The quantity of blood required is from five to twenty c.c., and in order to guarantee its sterility it should be drawn from a vein. The median cephalic or the median basilic vein is the one most commonly employed. The skin should be thoroughly cleansed with green soap, hot water, and a brush, and then with alcohol and ether. If the vein is very small or if absolute asepsis is to be guaranteed, it is wise to make a small incision down to the vein, but in general no contamination will result from the passage of the needle through the skin if the latter has been thoroughly cleansed.

The syringe should be one which can withstand the action of either dry heat at 150° C. for an hour or boiling in 1 per cent. sodium carbonate solution for fifteen minutes, as boiling in water without the addition of the alkali will not kill the resistant spores which are often present in syringes washed in tap water. The Lüer syringe is perhaps the best form for this purpose, though excellent instruments with asbestos packing are in the market.

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[1] *Burri:* Das Tuscheverfahren, Jena, 1909.

[2] See *Lenhartz:* Die Septischen Erkrankungen, Vienna, 1903; also *Rosenberger:* Path. Soc. of Phila., vol. vi, p. 158.

It is of advantage to have the needle of hardened platinum so that it can be burned off in a flame before plunging through the skin. It is convenient also to keep several Lüer syringes packed in large test tubes plugged with cotton and ready sterilized, the point of the needle being protected from injury by a mass of sterile cotton. Sterilization of the syringe with either carbolic acid or bichloride is inadmissible even though the antiseptic be washed out with sterile water.

In order to distend the vein an elastic bandage is placed about the arm, and the needle is plunged into the vessel against the direction of the blood current. In cachectic persons it is often necessary to find the vessels by palpation, as they can not be distended sufficiently to show under the skin. When the needle enters the lumen there will be a spurt of blood into the cylinder of the syringe and only gentle aspiration is necessary to obtain a considerable amount of fluid. If the needle is not sharp the vein frequently rolls under its point and escapes puncture.

The blood so obtained is now divided among a series of tubes containing melted agar at a temperature of 45° C. The amount in each depends somewhat upon the organism looked for, as, for example, the gonococcus requires a large percentage of blood serum in order to grow on agar, while an abundant strepto- or staphylococcus infection may give numerous colonies from only a few drops of the blood. The blood and agar in the culture tubes are mixed as quickly as possible and poured into Petri dishes and placed in the incubator. Some observers prefer to dilute the blood in a series of culture tubes or Erlenmeyer flasks containing a large quantity of sterile bouillon, when examining the blood of typhoid fever cases, the purpose being to avoid the bactericidal action of the shed blood; but, as a rule, the bacillus can be successfully isolated in agar cultures.

Anaerobic cultures should be made when infections by the Bacillus aerogenes capsulatus or other anaerobic bacilli are suspected. Such organisms have been most frequently found in puerperal sepsis.

When only a few colonies are obtained from a large quantity of blood, the question may arise as to the possibility of the colonies being derived from the skin or from the air. It is wise to discard all superficial colonies for this reason. If the colonies are deep and are on several plates, contamination may be ruled out. If a few colonies of two species are found a mixed infection may be present. Such mixed infections are, however, very rare, and if one or both of the organisms are such as may be derived from the skin the results must be interpreted with great caution.

For the details of the identification of the various infectious agents, the reader is referred to the standard text-books on bacteriology.

## RESULTS OF BLOOD CULTURES

In general infections with the **streptococcus pyogenes** the organism can be demonstrated in the blood in a considerable proportion of the cases, depending upon the site of the process. For example, in mycotic endocarditis due to the streptococcus, the organism can be obtained from the circulation in nearly all the cases, if not less than ten c.c. of blood are used and the examination is frequently repeated.

In puerperal sepsis only about one-fifth of the cases show the bacteria in the blood, that is, at least one bacillus to the fifteen or twenty c.c. of blood used in making the culture.

The specific organism can be isolated in from one-fifth to one-fourth of the cases of other types of sepsis due to the streptococcus.

The results obtained also vary with the observer, some reporting very few positive findings, others obtaining the organism in almost all cases. Cases with metastases in the joints or organs give a large proportion of positive findings.

Lenhartz reports five recoveries out of twenty cases in puerperal sepsis when the streptococcus had been found in the blood, while of seventeen other streptomycoses, three recovered.

The prognosis of streptococcus sepsis is not always bad. One-half of Bertelsmann's cases recovered, though other observers have noted that from 90 to 95 per cent. are fatal.

The streptococcus has also been found as a secondary infective agent in some of the eruptive fevers. It has been demonstrated in the blood of from 10 to 15 per cent. of persons suffering from scarlatina.[1] In the first few days of the disease and in the fulminating cases streptococci have not been found. The bacteriæmia is apparently an agonal phenomenon and the streptococci are not considered to be the specific incitants of the disease. The prognosis of those cases in which streptococci can be demonstrated in the blood is extremely bad.

The streptococcus has been found with considerable frequency in diphtheria and variola, chiefly in the fatal cases.

The same conditions apply to the **staphylococcus** infections as to the infections with streptococci as regards localization of the organisms favoring bacteriæmia; cases of endocarditis, osteomyelitis, or pyæmia due to the staphylococcus very often give positive blood cultures. In localized infections, however, the organisms are obtained in only a very small proportion of the cases examined.

The prognosis of a staphylococcus infection is much worse than that of a streptococcus. In seventeen cases Lenhartz reports sixteen deaths; Libman reports twenty-six cases with nineteen deaths.

The **pneumococcus** has been occasionally isolated from the blood in cases of sepsis due to this organism and not accompanied by pneumonia, the usual site for the process being the valves of the heart.

The pneumococcus can be demonstrated in the blood in a large proportion of cases of lobar pneumonia, but usually late in the disease and chiefly in fatal cases. Occasionally, however, the organism can be demonstrated as early as the second day after the chill. Rosenow[2] has obtained positive results in seventy-seven out of eighty-three cases examined, and in two others in which the cultures remained sterile, pneumococci could be demonstrated in smear preparations made directly from the blood. Prochaska[3] has also been able to demonstrate the pneumococcus in all cases he examined, some fifty in number.

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[1] *Jochmann:* Deut. Arch. f. klin. Med., 1903, Bd. lxxviii, p. 209 (Literature). *Hektoen:* Journal of American Med. Ass'n, 1903, vol. xl, p. 685.

[2] Trans. of the Chicago Path. Soc., 1903, vol. v, p. 265.

[3] Deut. Arch. f. klin. Med., 1901, Bd. lxx, p. 559.

Cole,[1] on the other hand, was able to find the coccus in only 30 per cent. of severe cases of pneumonia. Lenhartz obtained positive results in 30 per cent. of the eighty-three cases he examined.

One-half of the cases in whose blood pneumococci were demonstrated died.

As a rule, the number of bacteria present is not large when compared with the abundant findings of strepto- and staphylococci in the blood of septicæmia; but exceptionally from fifteen hundred to twenty-seven hundred [2] cocci to the cubic centimeter have been found before death. After death they may be present in enormous numbers, due to post-mortem growth.

The presence of the pneumococcus even in large numbers in the blood does not necessarily seem to be of unfavorable prognostic significance if we take the reports of Rosenow and Prochaska; but Cole and Lenhartz are inclined to regard the finding of the germ a matter of grave import.

As an aid to diagnosis, blood cultures in pneumonia are of comparatively slight value except in pneumonias of old people, where the physical signs are often obscure and the pneumococcus frequently invades the blood in considerable numbers. A frank case of lobar pneumonia can be diagnosed much more quickly and certainly from the physical signs than from blood cultures.

In a few cases the colon bacillus has been isolated from the blood in considerable numbers. The gonococcus has also been found.[3] Other bacteria which have been isolated from the blood during life are the tubercle bacillus, bacilli of anthrax and glanders, Friedländer's bacillus, Bacillus aerogenes capsulatus, Bacillus pyocyaneus,[4] and the paratyphoid bacillus. The bacillus of lepra has been demonstrated in smears. The Bacillus pestis is present in the blood of a large number of cases a few days before death, but not sufficiently early to replace other diagnostic methods.[5]

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[1] Johns Hopkins Hospital Bull., 1902, vol. xiii, p. 136.

[2] *Fraenkel:* Fest. f. v. Leyden, 1902, Bd. ii, p. 111.

[3] *Thayer* and *Blumer:* Bull. Johns Hopkins Hosp., 1896, vol. vii, p. 57. See also *Loeb:* Deut. Arch. f. klin. Med., 1900, Bd. lxv, p. 411.

[4] *De la Camp:* Zur Kenntniss d. Pyocyaneussepsis. Charité Annalen, 1904, Bd. xxviii, p. 92 (Bibliography).

[5] *Calvert:* Cent. f. Bakt. 1903, Bd. xxxiii, p. 247. See also *Atkinson:* Lancet, January, 1901, p. 231.

The influenza bacillus is said to have been obtained from the blood in a number of cases suffering from the grippe by Canon,[1] but the results have been questioned. Jeahle[2] claims to have been able to isolate the same bacillus from the blood of persons suffering from the eruptive fevers. In scarlatina the bacillus was found in twenty-two out of forty-eight cases, in measles in fifteen out of twenty-three, and in varicella in five out of nine. These results, however, need further verification.

The typhoid bacillus has frequently been obtained from the blood. Coleman and Buxton[3] have found that the bacillus has been demonstrated in 75 per cent. of five hundred and fifty-six cases personally observed or collected from the literature of the subject. Classification of the findings by stages of the disease shows:

1st week, bacilli found in the blood in 93 per cent.
2d week, bacilli found in the blood in 76 per cent.
3d week, bacilli found in the blood in 56 per cent.
4th week, bacilli found in the blood in 33 per cent.
Later than the fourth week, 18 per cent.

The preponderance of evidence seems to be that the typhoid bacillus disappears from the blood about the end of the third week in the majority of cases. Out of nineteen relapses the bacilli reappeared in sixteen. The bacteriological examination was positive with a negative Widal reaction in 10 per cent. of the five hundred and fifty-six cases.

### DIAGNOSTIC VALUE OF BLOOD CULTURES

The routine use of blood cultures in the study of cases of infection frequently furnishes results of great value from a diagnostic point of view if the specific organism can be isolated; but, unfortunately, the technical difficulties which underlie the isolation and identification of the bacterial parasites in the blood limit the application of the method to hospitals and well-equipped private laboratories.

It is a waste of time to inoculate a single culture tube with a few drops of blood and to attempt to draw conclusions from the morphological appearances of the bacteria so obtained or from their agglutinative reactions. The only way to avoid error is to plate large quantities of blood and then determine by subcultures on various media the biological characteristics of the organism.

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[1] Deut. med. Woch., 1892, p. 28.

[2] Zeit. f. Heilkunde, 1901, Bd. xxxii, p. 190.

[3] Proceedings of the N. Y. Path. Soc., N. S., 1904, vol. iv, p. 10. See also *Ruediger:* Trans. Chicago Path. Soc., 1903, vol. v, p. 187.

The agglutinative reactions used to identify the organism must be tested not only with serum derived from the patient, but also with serum of high valency produced by animal inoculations, to avoid errors due to the collateral agglutinins.

The possibility of contaminations derived from the skin renders the final determination of the etiological relationship of one of the pyogenic cocci a matter of great difficulty. The greater the care taken in the disinfection of the skin, the fewer will be the positive results obtained. The beginner will be disappointed by the large number of negative results which occur despite the most elaborate technique, even in cases of undoubted sepsis.

# IX. SERUM REACTIONS

## *A.* AGGLUTINATION REACTIONS

### GRUBER-WIDAL REACTION IN TYPHOID FEVER

The blood or blood serum derived from persons suffering from typhoid fever, or during convalescence from that disease, will, as a rule, cause the clumping and loss of motility of typhoid bacilli. This phenomenon may be observed when the serum is added in small quantities to the fluid containing the bacilli.

The exact cause of this clumping is still unknown, but it is assumed to be due to a reaction between the bacteria and an immune body or bodies (the agglutinins) circulating in the blood and formed by the action of the typhoid bacillus or its products, on the somatic cells during the course of the disease. The agglutinating substance causes *in vitro* the formation of clumps of typhoid bacilli when it has developed in sufficient concentration in the blood in a case of typhoid infection. The exact nature of the interaction between the bacilli and the immune substances has not been fully determined.[1]

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[1] An excellent résumé of the recent theories may be found in *Köhler:* Das Agglutinationsphänomen. Klin. Jahrbuch, 1901, p. 130 (Literature). See also *Widal* et *Sicard:* Annales de l'Institut Pasteur, 1897, t. xi, p. 353.

### TECHNIQUE

In order to obtain constant results in carrying out agglutination tests for typhoid fever it is necessary to use:

*a.* A standard culture of the typhoid bacillus.
*b.* Bouillon cultures of this bacillus grown for about fifteen hours at a fixed temperature, preferably 37° C.
*c.* Approximately equal numbers of bacilli from the fresh cultures for each test.
*d.* Bouillon of constant composition with a neutral reaction as determined by titration, using phenolphthalein as an indicator.

### PRESERVATION AND TRANSPLANTATION OF THE STANDARD CULTURES

Some standard culture of the typhoid bacillus should be employed of known agglutinating power, such, for example, as that of Pfeiffer, in order to obtain results which can be compared with those of others working the same bacillus. Different cultures react equally when mixed with the same agglutinating serum, and the best and sharpest reactions seem to be obtained by using an organism which has been cultivated through a number of generations on artificial media, rather than a fresh, virulent culture just obtained from a case of typhoid.

A series of subcultures of the standard bacillus should be made on slant agar, and, after burning off the cotton plugs, the tubes should be closed with paraffin or sealing wax. The bacilli in such tubes keep alive for an indefinite period if kept in a cool, dark place.

When necessary a fresh stock growth on slant agar should be made from one of the reserve tubes; and from this a tube of broth is to be inoculated and allowed to remain in the incubator for about fifteen to twenty-four hours. Some observers prefer a slightly lower incubator temperature than ordinary, say 34° to 35° C., or even room temperature, claiming that the motility of the growth is greater under these conditions, but this usage is not general. Transplantations should not be made from one broth tube to another, but always from the agar culture to broth, as pseudoclumping is apt to occur after several successive bouillon transplantations.

Pseudoclumping is also seen in some cultures which have grown in the incubator, and care should be taken to use only broth cultures in which the bacilli are distributed so as to produce an even clouding of the fluid, and also to avoid using any of the bacilli from the pellicle which may form on the surface. Different specimens of typhoid bacilli vary greatly in this regard, the formation of these clumps being due, apparently, to a simple mechanical adherence of the bacilli to each other.

Equally satisfactory results may be obtained by simply shaking up the moist bacterial growth, removed from the surface of the fresh agar culture, in broth or sterile physiological salt solution (0.85 per cent.), allowing the coarser particles to subside and using the mixture thus obtained; but careful controls are necessary to avoid errors, because of the pseudoclumping which may appear in such a mixture.

Cultures killed by formalin, thymol, or other preservatives, have been used by a number of observers with excellent results;[1] but there are some disadvantages inherent in this procedure, the chief of which is the absence of motility of the bacilli. The inhibition of the motility in live cultures is a most important factor in judging the completeness of the Widal test, and in differentiating pseudo-reactions.

It is always necessary to use an emulsion or culture containing about the same number of bacilli in performing an agglutination test, for the reaction may be considered as a titration method to determine the amount of agglutinin present in the blood, using typhoid bacilli as an indicator. If the number of bacilli present in the fluid tested is small, they may be clumped by a very small amount of a non-specific agglutinin, such as might be present, for instance, in normal human blood. If, however, the number of typhoid bacilli present in the serum is large, the normal agglutinins or the collateral agglutinins incited by other species of bacteria which are capable of affecting typhoid bacilli are absorbed in less proportion by the typhoid bacilli present and rendered relatively inert. As each typhoid bacillus has absorbed only a very small quantity of the non-specific or collateral agglutinin, it continues to be motile and is not agglutinated unless the serum is of considerable power.

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[1] *Pröscher:* Cent. f. Bakt., 1902, Bd. xxxi, p. 400. *Lion:* Münch. med. Woch., 1904, p. 909. *Gramann:* Serum Diagnosis in Typhoid by Ficker's Medium. Deut. med. Woch., 1904, p. 804. *Ruediger:* Journal of Infectious Diseases, 1904, vol. i, p. 236. *Meyer:* Ficker's Typhusdiagnosticum, Berl. klin. Woch., 1904, p. 166.

### OBTAINING THE BLOOD OR SERUM

The blood may be obtained by puncturing the finger or the ear with a Hagedorn needle, the puncture being rather deeper than that usually made for blood counts, as a number of drops are needed. The whole blood may be immediately diluted in a Thoma-Zeiss mixing pipette with culture broth or 0.9 per cent. salt solution; or several large drops may be allowed to collect in a small test tube or bottle and the clot allowed to separate from the serum, which will occur at the end of a few hours.

If much serum is required, it is easy to get large amounts by first distending the superficial veins of the arm by a bandage and then puncturing the vein directly through the skin, either with a Hagedorn or with a hypodermic needle; in the latter case the blood will flow through the lumen of the needle and may be received in a suitable vessel. Ten c.c. can easily be collected in this way. It is well to sterilize the needle after using, as typhoid bacilli may be present in the blood.

If the serum is to be kept any length of time it can be sealed up in the small capillary tubes used for vaccine lymph. Even if the tube is not sterile the agglutinating power is retained for considerable periods, varying in blood obtained from different individuals, and according to the method of preservation (cold or chloroform). After a month the strength of the serum may be considerably diminished, often to one-fourth or even one-tenth of the original titer.[1]

Blister fluid also gives the reaction and can be easily obtained by applying a small cantharides blister, one to two cm. in diameter, to any convenient portion of the body. In about twelve to eighteen hours enough serum will have collected in the blisters; and the fluid can be collected in capillary tubes and sealed, or diluted and used immediately.

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[1] *Puppel:* Cent. f. Bakt., 1900, Bd. xxviii, p. 877.

### DILUTION OF THE WHOLE BLOOD

This may be carried out immediately in a Thoma blood mixing pipette, as stated above, remembering that as the red cells form half the bulk of the blood the dilution should be only half that used when diluting serum. In other words, instead of diluting the blood 1 to 20, dilute it 1 to 10. In a drop of this 1 to 10 mixture the plasma which forms half the bulk is diluted 1 to 20, as the red cells may be considered for practical purposes as not giving up any of the agglutinating substances, as the solutions used in dilution are approximately isotonic and the corpuscles are not laked. When this 1 to 10 blood dilution is mixed with a drop of typhoid emulsion of equal size, the final dilution of the plasma is 1 to 40. Or, if preferred, the blood may be centrifuged before it has time to clot, and the clear plasma thus obtained diluted in the proper proportions.

### DILUTION OF THE SERUM

The dilution of serum which should be used in order to avoid diagnostic errors, is 1 to 60, but mixtures of 1 to 20 and 1 to 40 should be made at the same time and also examined. Broth or physiological salt solution should be employed as a diluent, for the reactions are not so delicate when distilled water is used, the reduction in the salt concentration probably affecting the result.[1] The pipette used should be most carefully cleaned after each case, for if a very small trace of a serum with high clumping power were left, it would contaminate the serum which follows and cause a false reaction. Some observers, therefore, prefer to use small capillary pipettes which can be thrown away after using.

A simple form of diluting apparatus can be constructed from ordinary glass tubing by drawing out portions into fairly fine capillary tubes. The tubes so prepared are calibrated for use either by marking point 1, corresponding to the space occupied by a small drop of fluid which has been drawn into the pipette, and then measuring off thirty-nine divisions further, and marking this 40; or by drawing up a known quantity of colored fluid and then forty times that amount, both quantities having been previously measured by accurate standard pipettes. Either a wax pencil or water-proof drawing ink may be used.

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[1] *Joos:* Mechanismus der Agglutination, Zeit. f. Hygiene, 1902, Bd. xl, p. 203.

The blood or serum is first drawn into this tube to the first mark, then the broth typhoid culture is drawn up to the point 20 or 40, and the contents of the pipette blown out upon a slide and covered with a cover glass.

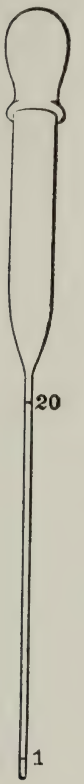

Fig. 63.—Pipette for Widal Reaction.

For the high dilutions now used it is better to purchase a number of small calibrated pipettes holding ten or twenty cubic millimeters. The serum is measured off in one of these and diluted with broth or salt solution delivered from another and larger pipette. One drop of the diluted serum and a drop of the broth culture of typhoid bacilli are mixed together on a cover glass and inverted on a hollow slide.

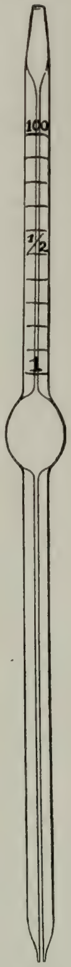

Fig. 64.—Durham's Diluting Pipette.

An excellent form of pipette[1] for making accurate dilutions of serum is shown in Fig. 64. The chief advantage of the instrument is that after each dilution has been made it is ready for further dilutions without cleansing. The method of employing the pipette is as follows: A rubber tube and mouthpiece having been slipped over the upper end, the pipette is filled with saline solution or broth up to the mark desired for the intended dilution. The pipette is now laid horizontally and the fluid to be diluted is allowed to flow into the pointed end from a fine capillary tube until the highest graduation is reached. The contents are then blown into a watchglass and used with an equal bulk of broth culture to carry out the agglutination. The expulsion of the fluid from the pipette secures the sufficient cleansing of the instrument for clinical work, as the inert saline solution following the serum to be diluted, washes out the capillary bore. It is not necessary to cleanse the instrument further, as tests with serum of high agglutinating power have shown that no error need be expected from traces of the original serum remaining in the tube.

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[1] *Durham:* A Pipette for Diluting Serum, Journal of Hygiene, 1903, vol. iii, p. 380.

### DRIED-BLOOD METHOD

Blood for Widal's test is often furnished, especially to municipal laboratories, dried on glass or paper. The accurate dilution of such dried blood is impossible. The best practical method is to put ten drops of water on the drop of blood, stir up and dissolve the crust, and consider such a dilution as 1 to 10. Then mix this 1 to 10 blood with a drop of equal size of a broth typhoid culture. The results obtained do not compare in accuracy or reliability with those furnished by the serum or blood dilution method.

### MICROSCOPIC REACTION

In order to observe the clumping of the bacteria a drop of the mixture is placed in the center of a cover glass and the latter inverted over a hollow ground slide, the edges of the concavity having been previously smeared with vaselin or immersion oil to prevent the cover sliding off and also to avoid evaporation and air currents in the chamber. It is well to warm the hollow slide slightly over a flame before putting on the cover, as otherwise the vapor from the warm broth culture will condense on the slide and interfere with the direct light from the condenser of the microscope. A dry lens of 4 to 5 mm. focus is the most convenient for this purpose, and artificial light is better than daylight. A control hanging-drop preparation of the culture should always be made first, to see

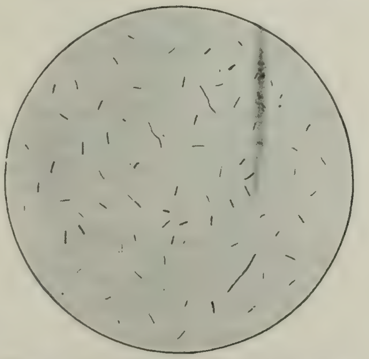
Fig. 65.—Control. The field is filled with actively motile bacteria.

if the bacilli are evenly distributed and not collected in pseudoclumps. Then the mixture is to be examined.

In order to find the bacilli, run the lens down as close as possible to the upper surface of the cover glass with the coarse adjustment, and preferably near the edge of the drop, then reduce the light to a minimum, and focus slowly upward with the fine adjustment. In this way there is no risk of plunging the lens through the cover glass and contaminating it.

### TYPES OF REACTION

The reaction is complete if the bacilli are clumped and immobilized in a few minutes. With a complete reaction the field of the microscope will be cleared of all motile bacilli in a few minutes. At the end of a few hours the outlines of the individual bacilli in a clump will become indistinct. In weak reactions, however, though clumping may have taken place, all the bacilli between the clumps may not become entirely immobilized, but retain some slight motion. If prompt clumping with *complete loss of motility* takes place at 1 to 60 in one hour, the blood is in 98 per cent. of the cases from a patient who has or has recently had typhoid.

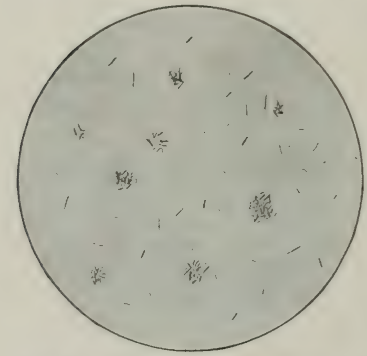

Fig. 66.—Imperfect Reaction. A few clumps are formed, but the motility of the bacilli persists.

If a prompt clumping takes place in a few minutes at 1 to 20 or 1 to 40, the disease is probably typhoid, but occasionally, especially in acute miliary tuberculosis, very suspicious pseudoclumps may be produced in a 1 to 40 or even in a 1 to 60 dilution. These clumps due to acute tuberculosis vary slightly from those of typhoid, as the bacilli retain their motility along the edge of the clump for a long time, and often cause the clump to rotate slowly in the fluid, while in typhoid motility ceases early (Figs. 67 and 68).

While in general it may be said that a positive agglutination reaction in fifteen minutes with serum diluted 1 to 10 is equivalent to that obtained by a 1 to 20 solution for half an hour, or a 1 to 40 for an hour, yet any one who has worked with the Widal reaction will have noted the fact that the serum from certain cases of typhoid fever gives no agglutination at 1 to 10 or 1 to 20, but causes marked clumping when the serum is still more diluted, for example, to 1 to 60 or 1 to 100. The exact explanation of this phenomenon is not as yet known. By some [1] it is considered to be due to the rapid paralysis and subsequent lysis of the bacteria by the action of the bacteriolytic substances in the blood; others have assumed the presence of proagglutinoid substances which unite with the agglutinable bacteria and prevent the action of the true agglutinins.[2] The practical fact which we may derive from the phenomena mentioned above is that all Widal tests should be made in a series of dilutions, using at least 1 to 20 and 1 to 60 for clinical diagnosis. If the reaction even in early cases is inhibited at 1 to 20, further tests will at times show characteristic clumping at 1 to 60.

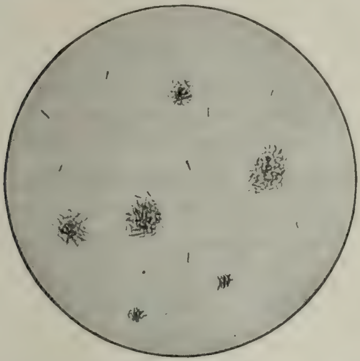

Fig. 67.—Complete Reaction. The bacteria are collected in large masses, and the few which remain between the clumps are not motile. The outlines of the bacilli are not sharply marked in the hanging-drop; but if the preparation is allowed to dry on the cover glass and then stained, the bacteria will be seen to have preserved their normal shape.

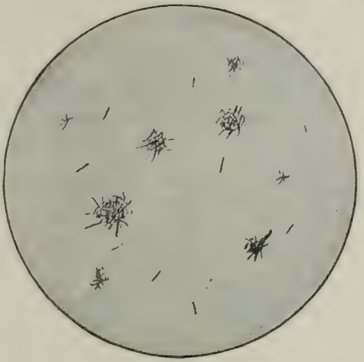

Fig. 68.—The Type of Agglutination seen in Tuberculosis. Clumping takes place, but the bacilli forming the clumps retain their motility and, projecting from the periphery of the mass of agglutinated organisms, give a rotary motion to the clump. The bacilli which are not agglutinated retain a well-marked motility.

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[1] *Volk* u. *de Waele:* Wien. klin. Woch., 1902, p. 1305. *Hiss:* (oral communication).

[2] *Eisenberg* u. *Volk:* Wien. klin. Woch., 1901, p. 1221.

### MACROSCOPIC REACTION

The method just described for observing the process of agglutination in a hanging-drop preparation under the microscope, is that most generally used for diagnostic purposes. It is also especially convenient because the reaction can be carried out with small quantities of serum and requires only simple apparatus. The phenomena of agglutination as observed under the microscope are sharp and the differences between a complete Widal reaction and one of the pseudo-reactions may be noted after very slight experience. For scientific purposes, however, it is often important to use another technique, the so-called macroscopic method, performed in small test tubes, in which the agglutination is observed by a hand lens.

The technique is as follows: About two c.c. of blood should be obtained and allowed to coagulate in a small bottle or other suitable receptacle lying horizontally. After a firm coagulum is formed, the bottle is placed vertically in a cool place and the serum which is extruded from the clot is allowed to collect at the bottom. After twenty-four hours a considerable bulk of serum is ready for dilution. A measured amount, for example, one-half c.c., is placed in a small test tube[1] and diluted with nine and one-half c.c. of physiological salt solution, thus giving a final dilution of 1 to 20 when mixed with an equal bulk of bacterial emulsion. Similar dilutions are then made from this of 1 to 40, 1 to 60, and 1 to 100. To each of the series of test tubes containing the diluted serum is added an equal bulk of an emulsion of typhoid bacilli in physiological salt solution.[2] This emulsion is made by pouring the saline fluid into a culture tube containing a fresh growth of typhoid bacilli on slant agar. The bacteria are then mixed with the salt solution by rubbing the culture off the surface of the agar with a platinum loop, and after the coarse particles have settled the bacterial emulsion is ready to be added to the test tubes containing the serum diluted for the reaction.

All of the glass utensils employed in the macroscopic agglutination method should be sterilized by dry heat to 150° C. Pipettes used in measuring out typhoid emulsions should have a cotton plug inserted in the lumen a few centimeters below the upper end. This prevents contamination of the culture by the saliva and diminishes the risk of mouth infection should the fluid be drawn too high. A stiff rubber bulb may also be used to aspirate the fluid into the pipette. The bulb should have a perforation which is closed by the finger while suction is being made.

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[1] Convenient tubes for this purpose measure 11 cm. in length with a lumen of 7 or 8 mm.

[2] This is preferable to a bouillon culture, as there is less nutrient material present, and the clumping is not obscured by a rapid growth of bacteria in the medium.

Another method which avoids the pipetting of typhoid cultures —a procedure which is always hazardous—is to make up dilutions of the serum, 1 to 40, 1 to 60, and 1 to 120, and then inoculate each one of the test tubes containing this serum with a loopful of typhoid bacilli scraped from the surface of a twenty-four-hour agar culture. To do this the loop carrying the mass of bacteria should be rubbed against the inner surface of the test tube just above the fluid, and then by gently inclining the tube the emulsion can be rubbed up with small quantities of the diluted serum, and finally the whole thoroughly stirred with the loop. The tubes containing the serum and bacteria are placed in the incubator for one hour. At the end of this time they should be examined with a hand lens, holding the tube so that light falls obliquely through it.

The granular appearance of the flocculi of the agglutinated bacteria can be easily recognized with the naked eye or with a hand lens by an experienced observer, but the beginner should always learn to recognize the agglutination by first working with a typhoid serum of known agglutinating power.

Control tubes of the emulsion of the bacilli, one of which should be placed in the incubator at the time of making the test, should remain opalescent and equally cloudy throughout during the time of observation. The deposit which may form at the bottom of the control tubes or those containing the serum has nothing to do with the agglutination.

In working with a bacterial culture, the limits of whose agglutinating reactions are not known, it is also necessary to control these tests with normal serum to determine the limits of what may be called the normal agglutinins. Some typhoid bacilli seem especially sensitive to the normal agglutinating power of the serum, and the blood of some healthy individuals has been found to agglutinate these bacilli even as high as 1 to 40 in an hour.

## TIME AND CONSTANCY OF APPEARANCE OF THE WIDAL REACTION

In a few cases the agglutinating substances appear very early in the disease, so far as the day of the disease can be judged from the clinical symptoms, for it must be remembered that what is loosely termed the first week of a typhoid fever may in reality be much later in the course of the disease. The custom in vogue in hospital work of considering the day upon which the patient takes to his bed as the beginning of the disease may be sufficient for practical purposes, but not for accurate statistics on the time of appearance of the Widal reaction. It is much better to select the first appearance of the eruption as denoting about the tenth day of the disease, as Higley [1] has suggested, and compute the beginning of the first week by this means. Inasmuch as the eruption is not constantly present, this means of estimating the course of the disease is not always applicable, and we must use the history of the prodromal stage to obtain even approximate results.

In considering the first appearance of the reaction a great deal depends upon the dilution used. One observer,[2] using 1 to 50 dilution for two hours, obtained reactions in the first week in about 35 per cent. of his cases, while the writer, using 1 to 60 for one hour, obtained positive results in not over 10 per cent. of the cases. A considerable number of these cases, however, gave a good agglutination at 1 to 20 in an hour; but such reactions can not always be relied upon, as they may occasionally be found in normal blood, in anæmia, tuberculosis, pneumonia, and sepsis.

In the second week the reaction is present in about 80 per cent. of the cases, using a dilution of 1 to 60 for an hour, while during the fourth week the remaining 8 or 9 per cent. give the reaction. In the writer's experience the reaction at this dilution is absent from not more than 1 or 2 per cent. of typhoid fever cases *when the blood is frequently tested* during the course of the disease. If only one or two examinations are made, the number of positive reactions obtained falls much lower owing to the fact that the agglutination appears to this degree in some cases of typhoid only for a few days and then becomes much weaker.

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[1] Proceedings of the N. Y. Path. Soc., 1903, N. S., vol. iii, p. 87.

[2] *Tobieson:* Zeit. f. klin. Med., 1901, Bd. xliii, p. 147.

Gwyn's[1] results show the high average of 99.6 per cent. of positive reactions obtained during the four weeks of the fever, while Libman informs me that using dried blood and diluting 1 to 20, he has never seen a typhoid case which did not give the reaction at some time during the course of the disease. In children, Gerschel,[2] using dried blood and a dilution of 1 to 20, reported that 96 per cent. of the cases gave a positive reaction. Tests of large groups of cases, chiefly by the dried-blood method,[3] reported by municipal laboratories, show that the reaction is obtained in about 95 per cent. of cases considered as typhoid from the clinical symptoms.

### PERSISTENCE OF THE WIDAL REACTION DURING CONVALESCENCE

The blood of persons who have had typhoid retains its agglutinating power for a considerable time, but the period varies a good deal in individual cases.[4] In children the reaction does not persist so long as in adults.

The average time during which a 1 to 40 agglutination may be obtained is probably less than a month, and the writer has seen a number of cases in which the reaction has disappeared at the end of three to six weeks, though the agglutinative power of the blood had been high during the course of the disease. There is no evidence, however, that the severity of the attack or the degree of the agglutination during that period determines the prolongation of the reaction.

Cases in which the reaction persists for months or years are probably due to a persistence of the infection. The gall bladder frequently seems to be the site of lodgment of the bacilli which continue the inciting process. It has also been shown that in many cases of typhoid fever bacilli pass into the bile and can be frequently demonstrated in the gall bladder at autopsy,[5] or even during or after convalescence. Cases have been published of the isolation of typhoid bacilli from the gall bladder in patients suffering from cholelithiasis, one seven years, the other eighteen years after an attack of typhoid fever.[1] The Widal was positive in both. A positive reaction has been found five and sixteen months after a typhoid osteomyelitis.[2]

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[1] Johns Hopkins Hosp. Bull., 1900, vol. viii, p. 385.

[2] N. Y. Medical Record, 1901, vol. lx, p. 891.

[3] *Abbott:* Annual Rep. Philadelphia Bureau of Health, 1899, p. 199; ibid., 1900, p. 92; ibid., 1901, p. 107.

[4] *Courmont:* Semaine Médicale, 1897, vol. xvii, pp. 69 and 105.

[5] In 70 per cent. of the cases, *Pratt:* Am. Jour. Med. Sci., 1901, cxxii, p. 584.

### AGGLUTINATION REACTIONS IN ICTERUS AND MIXED INFECTIONS

The blood of some patients suffering from icterus has the capacity to agglutinate typhoid bacilli in a dilution of 1 to 40 at the end of one hour. This phenomenon is not constant, and is found in types of icterus due to different conditions. For example, the writer has noted such agglutination in the blood of one case of hepatic cirrhosis with slight jaundice but without cholecystitis, as was shown post mortem. On the other hand, he has frequently failed to obtain an agglutination 1 to 40 in cases of intense jaundice.[3] Zupnik has called attention to the frequency with which the Widal reaction may be obtained in Weil's disease though no typhoid infection exists.[4] Concentrated human bile has been shown[5] to be capable of agglutinating typhoid bacilli in a 1 to 80 dilution, or even higher; but the injection of moderate amounts of bile into the circulation or the production of icterus by ligation of the common duct has not, in animals at least, resulted in the production of high degrees of agglutinating power.

The present view is that the patient showing such an agglutination has had typhoid fever at some previous time, and that the gall bladder has been infected by the typhoid bacilli with the production of a chronic cholecystitis. This inflammation may incite the formation of gall stones or cause jaundice by inducing chronic inflammatory changes in the common duct with stenosis. The stimulation of the production of agglutinating substances is then continued by the action of the bacteria, either in the rôle of a general infective agent, or by setting up a local process in the gall bladder.[1]

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[1] *Cushing:* Johns Hopkins Hosp. Bull., 1898, vol. ix, p. 91. *Miller:* ibid., p. 95. *Hunner:* ibid., 1899, vol. x, p. 163 (Literature). *Osler:* Trans. Am. Assoc. of Phys., 1897, vol. xii, p. 378.

[2] *Elsberg:* N. Y. Med. Record, 1897, vol. li, p. 510 (1 to 8 reaction in 15 minutes with dried blood). *Thayer:* Johns Hopkins Hosp. Bull., 1897, vol. viii, p. 54.

[3] See also *Gilbert* et *Lippman:* Compt. Rend. d. la Soc. d. Biol., 1903, p. 1705.

[4] Zeit. f. Heilkunde, 1901, Bd. xxii, p. 334. *Eckhardt:* Münch. med. Woch., 1902, p. 1129.

[5] *Köhler:* Das agglutinations Phänomen, Klin. Jahrbuch, 1901.

The fact that typhoid bacilli have been isolated from the gall bladder years after an attack of fever, as stated in the previous section, and that gall stones frequently contain bacilli, especially those of the colon group, give color to this theory.

Primary typhoidal cholecystitis without a history of a previous typhoid fever is also not unknown, so that some of the reactions obtained in jaundiced patients may be due not to the jaundice, but to a typhoid infection with cholelithiasis and blocking of the common duct.[2]

An interesting case belonging to this group has been seen by Norris.[3] The patient suffered for five years with gall-stone colic, the last attack being accompanied by jaundice. An attack of typhoid fever followed shortly after. The blood gave the Widal reaction and the typhoid bacillus was isolated from the stools. No further attacks of gall-stone colic or jaundice have occurred since convalescence from the typhoid fever. Possibly this case had a primary infection of the gall bladder with typhoid bacilli followed by the production of gall stones, and a secondary infection of the intestinal tract inciting the typhoid fever.

Since the discovery of "collateral agglutinins," the suggestion has been made[4] that the agglutinative reaction is produced by the general or local infection of the patient by other bacilli, especially by members of the colon-typhoid group. Such "collateral agglutinins" are most frequently met with in infections with the different members of groups of closely related bacteria, but may even occur in infections with wholly unrelated bacteria, such as the proteus, the tubercle bacillus, and even the staphylococcus. It is not uncommon, as has been previously stated, to meet with a 1 to 40 agglutination closely simulating a Widal reaction in cases of pneumonia, miliary tuberculosis, and sepsis.[5]

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[1] For an interesting case of this type, see *Browne* and *Compton*, Lancet, 1903, vol. i, p. 1798.

[2] *Stewart:* Am. Medicine, 1904, vol. vii, p. 1018 (Bibliography.)

[3] Oral communication.

[4] *Köhler:* Münch. med. Woch., 1903, p. 1379. *Steinberg:* ibid., 1904, p. 469.

[5] *Lommel:* Widal Reaction 1 to 80 in Case of Puerperal Sepsis, Münch. med. Woch., 1902, p. 314; *Megele:* Widal Reaction in a Case of Liver Abscess due to Staphylococcus, Münch. med. Woch., 1903, p. 598; *Lubowski* u. *Steinberg:* Deut. Arch. f. klin. Med., 1904, Bd. lxxix, p. 396.

Such reactions are, however, not regularly found in these conditions, and animal experiments have shown that they can not be constantly produced artificially. Some individual sensibility to the action of the bacterial poisons may underlie the phenomenon.

Thus it is seen that it is often impossible to decide whether the agglutinins causing the clumping of the typhoid bacillus are due to the specific substances produced by that bacillus or to collateral agglutinins due to infection with some other organism. Sometimes the isolation of the specific organism by cultures from the blood or stools will answer the question. If the infection is due to some other organism, advantage can be taken of the absorption of the specific agglutinins by the bacteria producing them.[1]

If, for example, a streptococcus infection is suspected though a Widal reaction is given, a considerable quantity of the patient's serum should be obtained and a number of loops of a fresh streptococcus culture added, and the whole set aside for twelve hours at incubator temperature. The clear supernatant serum is then poured off and the process repeated. The Widal reaction is then carried out using typhoid bacilli. If the agglutinins are due solely to the streptococcus, they will all be absorbed by that organism and the typhoid bacilli will not be clumped. If the infection, however, is due to typhoid bacilli, only the agglutinins affecting the streptococcus will be absorbed and the typhoid bacillus will clump at practically the original dilution.

The problem is the same when agglutination reactions are obtained with two bacilli. For example, it is not uncommon to find the serum of a characteristic clinical case of typhoid fever clumping both the typhoid bacillus and B. enteritidis (Gärtner) at high dilutions. If the clumping of the Gärtner bacillus is due to collateral agglutinins produced by the typhoid infection, saturation with typhoid bacilli will remove all agglutinating power from the serum. Great care must be used in applying the method generally, as the following illustrations will show. If the clumping is due to a third bacillus which is not suspected, saturation with the typhoid bacillus will remove the agglutinins for that organism and then the serum will clump the B. enteritidis; or if the B. enteritidis has been used to extract the specific substances, the typhoid bacilli will be clumped. In neither case is the organism used in saturation the inciting agent of the disease, and recourse must be had to blood cultures to make a diagnosis during life. Such complex agglutinins are rarely of high degree, and if the serum is well diluted the organism causing the disease will generally be clumped while the others are not affected.

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[1] *Castellani:* Zeit. f. Hygiene, 1902, Bd. xl, p. 1.

These tests have not as yet been made on a sufficiently large amount of clinical material to warrant final conclusions; but in the few cases reported they have been satisfactory from a diagnostic point of view.[1]

### DIAGNOSTIC VALUE OF THE WIDAL REACTION

The late appearance of the Widal reaction in dilutions which can be considered as absolutely diagnostic of the disease, makes it generally of comparatively little value in the early diagnosis of an active and well-marked case of typhoid fever, as a diagnosis under these conditions can be made from clinical symptoms, and often by making cultures from blood or stools, much earlier than from the serum reaction. If, however, the clinician is himself in a position to observe the agglutination test and to interpret it, very suggestive results may be obtained during the first week by using 1 to 20 dilutions for an hour, though such agglutination reactions can not be considered as positively diagnostic of typhoid fever; they are merely highly suggestive of that condition. Used in the same way, an agglutination of 1 to 40 in an hour furnishes a probable diagnosis of typhoid fever in over 90 per cent. of the cases and is therefore useful when the limitations of the reaction are understood. A 1 to 60 agglutination in an hour with complete loss of motility may be considered as practically diagnostic of typhoid fever.

The Widal reaction is of especial value in the diagnosis of obscure or ambulant cases of typhoid fever where the clinical symptoms may not be characteristic at any time during the course of the disease. It is also of value in the diagnosis of typhoid fever in infants, and especially in those cases in which the symptoms referable to intestinal lesions are less prominent features of the disease and the clinical picture is dominated by the symptoms of a general infection.

The reaction is also useful in distinguishing between typhoid fever and miliary tuberculosis, if sufficient care be used in differentiating the agglutination due to infection by the tubercle bacillus and that by the typhoid. A differential diagnosis between typhoid fever and the obscure intestinal infections lasting only a few days, which are often seen during the summer months, can often be made by the Widal reaction.

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[1] For a recent summary of the subject, see *Stober:* Journal of Infectious Diseases, 1904, vol. i, p. 445.

## SERUM DIAGNOSIS BY MEANS OF BACTERICIDAL ACTION

The use of the bactericidal action of the blood serum of typhoid-fever patients has been suggested as a means for the diagnosis of that disease, but the procedures required are so complicated that the method must necessarily be limited in its use. It may be used in cases where no Widal reaction is given and when it has been impossible to isolate the typhoid bacillus from the blood or stools.

The method[1] is as follows: Half a cubic centimeter of a twenty-four-hour bouillon culture of typhoid bacilli which has been diluted 1 to 5,000, and half a cubic centimeter of either normal human or rabbit serum diluted 1 to 10 with 0.85 per cent. salt solution, are mixed in a test tube with one c.c. of the dilution of typhoid serum to be tested. This serum should be diluted with 0.85 per cent. salt solution, 1 to 2,000, 1 to 4,000, etc., up to 1 to 32,000. This necessitates the preparation of five different test tubes, one for each dilution. A control tube should also be prepared with one c.c. of normal salt solution, one-half c.c. of the dilute culture, and one-half c.c. of the normal rabbit serum. The "complement"[2] of the typhoid serum to be tested must be removed by heating the fluid to 56° C. for half an hour, although when working with dilutions over 1 to 10,000 it may be omitted, as the amount of "complement" is too small to be of influence, and as in a considerable proportion of the cases so far observed the bactericidal action was noticed in 1 to 50,000 dilution, a preliminary test may be made at this point.

After the tubes are prepared they are placed at 37° C. for three hours and then, with the controls, mixed with melted agar and poured into plates, which can be examined for growth twelve to twenty-four hours later. It is not necessary to enumerate the colonies, the degree of bactericidal action being determined by comparing the plates with controls. A marked reaction occurs when only a few colonies develop. The reaction has been noted as early as the eighth day of the disease in a 1 to 4,000 and even in a 1 to 40,000 dilution. It has also been very well developed in cases not giving an agglutination 1 to 40. Its chief value is in what may be called retrospective diagnosis, when it is important to decide whether a person has had typhoid fever at some previous time, as the bactericidal action often persists for more than a year after the disappearance of the agglutinative power of the blood.

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[1] *Stern* u. *Korte:* Berl. klin. Woch., 1904, p. 213.

[2] This term is used to designate a substance (also called alexin) which is present in the blood of both normal and immunized animals. It is this substance which, when joined with the bodies produced by immunization with typhoid bacilli, for example, enables the immune body to exert its lytic action on these cells.

## *B.* AGGLUTINATION REACTIONS IN DISEASES OTHER THAN TYPHOID

### PARATYPHOID INFECTIONS

A certain number of cases clinically resembling typhoid fever do not give an agglutinating reaction with typhoid bacilli. From some of these cases a group of bacilli has been isolated, the members of which resemble typhoid bacilli in some of their cultural characteristics. They differ from the latter, however, especially in the formation of gas in glucose bouillon and by their serum reactions. The blood serum of a patient suffering from infection with one of these varieties will agglutinate the species isolated from the patient's blood in high dilutions; but not all cases of paratyphoid fever will agglutinate equally well with paratyphoid bacilli isolated from different epidemics, though two general groups may be distinguished by cultural and agglutinative properties. It is necessary, therefore, in case no agglutination is obtained with a typhoid bacillus, to secure members of both groups of the paratyphoid bacilli as isolated by different observers, and carry out agglutination tests with each one of the series. A high agglutination with any one of the varieties used indicates a probable infection with that variety of bacillus.[1]

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[1] See for further details on this subject, *Schottmüller:* Zeit. f. Hyg., 1900, Bd. xxxvi, p. 368; *Coleman* and *Buxton:* Am. Jour. Med. Sci., 1902, vol. cxxiii, p. 976; *Johnston:* ibid., 1902, vol. cxxiv, p. 187; *Hewlett:* ibid., p. 203; *Longcope:* ibid., p. 209; *Libman:* Jour. of Med. Research, 1902, N. S., vol. iii, p. 168; *Bruns* u. *Kayser*, Zeits. f. Hyg., 1903, Bd. xliii, p. 401; *Korte:* ibid., 1903, Bd. xliv, p. 243; *Stober:* Jour. of Inf. Dis., 1904, vol. i, p. 445.

### INFECTIONS DUE TO THE BACILLUS COLI COMMUNIS

General infections by the B. coli communis show a moderate agglutinating power in the blood for fresh twenty-four-hour cultures of the organism. The number of observations made is still too few to allow conclusions of diagnostic value to be drawn from the results.[1]

Colon bacilli, which have been assumed by some observers to be the cause of acute intestinal infections, have been found to give, especially in children, a very moderate agglutinative reaction in the blood, generally not over 1 to 10 or 1 to 20 at the end of an hour. This reaction is most marked when carried out with a bacillus isolated from the intestine of the patient furnishing the agglutinating serum. The reaction, however, is not constant, and is rarely sufficiently high to avoid confusion with the normal agglutinating power of human blood serum, which often acts upon the colon bacillus in a dilution of 1 to 10.

### INFECTIONS DUE TO MEMBERS OF THE DYSENTERY GROUP OF BACILLI

This group of bacilli contains at least three separate species, differing from each other in their agglutinations and reactions when grown upon sugar media. The bacillus originally isolated by Shiga [2] from cases of endemic dysentery in Japan has been shown to give a macroscopic agglutination with the blood of these patients in dilutions even as high as 1 to 40 or 1 to 60 in an hour.

Pilsbury [3] found that the Shiga type of dysentery bacillus occasionally agglutinates 1 to 100 in three hours, with the blood of persons not suffering from dysentery, but that this agglutinating power does not exist in the blood of infants. He concludes that a prompt reaction under two hours with the Shiga type of bacillus in dilutions of 1 to 20 in children, and 1 to 50 in adults who have not recently suffered from intestinal disease, is suggestive of an acute infection with the Shiga bacillus.

The Flexner [4] type of bacillus has also been found to agglutinate in low dilutions with the blood of patients from whose stools this bacillus has been isolated.

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[1] See for further details on this subject, *Sailer:* Diagnosis by Means of the Serum of the Blood, Univ. of Penna. Med. Bull., 1902, vol. xv, p. 204.

[2] Deut. med. Woch., 1901, p. 741.

[3] N. Y. Medical News, 1903, vol. lxxxiii, p. 1078.

[4] Bull. Johns Hopkins Hosp., 1900, vol. ii, pp. 39 and 231.

Wollstein[1] found that an agglutination reaction was absent in twelve cases out of thirty-three in which the Flexner type of organism was isolated from the stools. "Good reactions" (no time or dilution given) were present in twenty-one, most frequently from the fourth to the eighth day of the disease.

The third variety, the Bacillus "Y" of Hiss[2] has not as yet been tested sufficiently to determine its agglutination limits in clinical cases of dysentery. The reaction only appears late in the course of the disease and low agglutinations were observed in fatal and also in very mild cases.

It is evident that, in the present state of our knowledge, agglutination reactions can not be used in the diagnosis of dysentery until the relations between the disease and the different groups of bacilli are more satisfactorily determined.

### CHOLERA

A moderate number of tests have been carried out on the agglutinating reaction of serum from cholera patients on the specific bacillus, and such reactions have been found to appear at a fairly early period in the course of the disease, and rise as high as 1 to 40. Some cases, however, do not give the reaction. The bacilli should be used in a salt solution suspension and not in broth culture, as pellicle formation is very apt to occur in a fluid medium.

A diagnosis can be made earlier and more certainly by the isolation of the bacilli from the stools and testing them with an immune serum of high agglutinating power.[3]

### PLAGUE

An agglutination of the plague bacillus has been observed beginning with the second week of the infection and rising later even as high as 1 to 40. The reaction, however, is of little practical value, since the diagnosis can be made much earlier by the cultural isolation of the bacillus from the bubo or by the inoculation of susceptible animals.[4]

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[1] Jour. of Med. Research, 1904, N. S., vol. v, p. 11.

[2] *Hiss* and *Russell:* Proc. N. Y. Path. Soc., 1902, N. S., vol. ii, p. 157; also Medical News, 1903, vol. lxxxii, p. 289.

[3] See *Kolle* u. *Gotschild:* Zeit. f. Hygiene, 1903, Bd. xliv, p. 1.

[4] *Martini:* Zeit. f. Hygiene, 1902, Bd. xli, p. 159.

### MALTA FEVER

The serum of patients suffering from Malta fever usually gives a marked agglutination with the Micrococcus melitensis. The reaction has been seen to occur in a dilution as high as 1 to 50, or in some cases 1 to 300.[1] Using emulsions of dead bacteria in physiological saline solutions, the agglutinating power of the serum may rise to 1 to 1,000 or more, if the mixture is left for twenty-four hours at room temperature.[2]

### TUBERCULOSIS

The serum of a tuberculous patient is capable of agglutinating tubercle bacilli;[3] but inasmuch as negative reactions are frequently met with and as agglutinations of B. tuberculosis can be obtained with normal blood serum, the actual value of the reaction from a clinical point of view is still much in doubt.[4]

### PNEUMONIA

It has been shown by Bezancon and Griffon[5] that normal undiluted blood serum when inoculated with a culture of the pneumococcus is diffusely clouded by a growth of the bacteria at the end of about fifteen hours at 37° C. In the serum of persons suffering from pneumonia the growth at the end of fifteen hours does not cloud the fluid, and the bacteria can be found in the sediment which collects in the lower portion of the test tube in the form of masses and chains, either in fresh or stained preparations. The agglutination can usually be easily distinguished by the eye or with a hand lens from the clouding in the normal serum. The reactions have been observed as high as 1 to 50, but not in all cases of pneumonia.

Wadsworth[6] has modified this procedure and has obtained results which are fairly constant by using a concentrated emulsion of the pneumococci. He finds it essential to grow the organism in a 1 per cent. peptone broth made from meat infusion which has been carefully neutralized before boiling.

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[1] *Kretz:* Wien. klin. Woch., 1897, p. 1076.

[2] *Basset-Smith:* Brit. Med. Jour., 1902, p. 861.

[3] *Arloing* et *P. Courmont:* Gaz. des hôp., 1900, vol. lxiii, p. 1467.

[4] See *Loeb:* Trans. Chicago Path. Soc., 1902, vol. v, p. 141.

[5] Ann. de l'Inst. Pasteur, 1900, vol. xiv, p. 449.

[6] Jour. of Med. Research, 1903, vol. x, p. 228.

Two hundred c.c. of fluid in a flask is inoculated from a culture of the pneumococcus, and at the end of thirty-six hours the fluid is centrifugalized, the clear fluid decanted, and the sediment shaken up with fifteen c.c. of 0.85 per cent. solution salt. Dilutions of the sera to be tested are then made in small test tubes and observed for about twelve hours at 37° C. Marked reactions may be complete in five or six hours.

The technical difficulties of the procedure are so great that the method can not be used at present for routine diagnosis. The physical signs of the disease, also, precede the reaction by some days, so that the demonstration of the agglutination is of importance only in very obscure cases. More satisfactory results from a diagnostic point of view may often be obtained by making blood cultures.

## *C.* PRECIPITIN REACTIONS IN BLOOD SERUM

### NATURE OF THE REACTION

The experiments of Bordet,[1] Tschistovitch,[2] and Nolf,[3] have shown that when an animal is injected with the blood or blood serum of another animal, new bodies are formed in the blood serum of the inoculated animal. These bodies give rise to precipitates when mixed with the serum of the animal with whose blood the injections were made.

The same phenomenon has been observed after the injection of a large variety of proteid substances, both animal and vegetable. The test animals injected with these substances furnished a serum which precipitated a solution of the substance injected.

These precipitation reactions were originally considered as specific for the variety of albumin injected or for the species of animal or plant from which the albumin was obtained, but further investigations have shown that not only are the reactions not specific for the different types of albumin, but they are not strictly specific for the given animal species, except under definitely limited conditions. They may be considered rather as generic in their action—i. e., precipitins obtained by injecting human blood into the peritoneal cavity of a rabbit will give rise to precipitates not only in human blood, but also in the blood of cognate species of primates, the reaction being most marked in the higher groups of apes and appearing but faintly in the more distantly related species. In the same way, sera obtained after injecting beef blood into an animal will give a slight precipitate in sheep and goat blood.[1]

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[1] Ann. de l'Inst. Pasteur, 1899, vol. xiii, p. 240.

[2] Ibid., p. 413.

[3] Ibid., 1900, vol. xiv, p. 299.

It is evident that this reaction may be of great value in the study of blood stains from a medico-legal point of view. It has also been applied to the investigations of albuminous substances which appear in the urine either as the result of an inflammatory process in the kidney or as a filtrate through the kidney epithelium following the ingestion of large quantities of proteid substances (so-called dietetic albuminuria).

### PRODUCTION OF THE ANTISERUM

The fluid injected to produce an antihuman serum may be blood obtained either from the placenta or at autopsy from a case not dying of an infectious disease. It is not absolutely necessary to inject the blood corpuscles to obtain a specific reaction as the serum contains the substances which incite the formation of the precipitating body; so that instead of blood or blood serum, exudates or transudates from the pleural or peritoneal cavities may be employed, if these fluids are sterile. If the reaction is to be employed for medico-legal purposes, however, it is generally considered safer to use whole blood.

In order to obtain the serum to carry out the precipitation tests it is necessary to inject the animals selected with considerable quantities of blood or blood serum. The animal employed is usually the rabbit, although Ewing[2] has found that the chicken is excellent for the purpose. Where absolute differentiation between human blood and blood of the higher apes is required, the suggestion has been made that the monkey be used, as the reaction is then more specific. As a rule, however, the antiserum produced in the rabbit is satisfactory for all purposes. A number of animals should be injected as only a few give serum of high potency despite the frequent exhibition of large quantities of blood.

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[1] A great part of our knowledge of the precipitating action of antisera upon the blood of various animals we owe to *Nuttall*, whose book on Blood Immunity and Blood Relationship (Cambridge, 1904) covers the subject in a most thorough manner and contains a very complete bibliography. See also *v. Dungern:* Die Antikörper, Jena, 1903, and *Rostoski:* Zur Kennt. d. Präcipitine, Würzburg, 1902. Full technical details, as well as a complete bibliography to 1910, can be found in *Uhlenhuth* und *Weidanz:* Prakt. Anleit. zur Ausführung des biologischen Eiweissdifferenzierungsverfahrens, Jena, 1909, and *Leers:* Die forensische Blutuntersuchungen, Berlin, 1910.

[2] *Ewing* and *Strauss:* New York Med. News, 1903, vol. lxxxiii, p. 871.

### TECHNIQUE OF INJECTION

The rabbit to be injected is tied down on an animal holder or held by an assistant. The abdomen is scrubbed with soap and water and the hair shaved over a small area of the left portion of the abdomen. It is well to cleanse the skin further by the application of lysol or alcohol and 1 to 1,000 sublimate solution.

The serum or blood to be injected is drawn into a syringe provided with a needle of large caliber, and after making a slight incision through the skin, the needle of the syringe is entered obliquely through the abdominal wall. The needle should be inserted through the muscles of the abdomen very gently, and the peritoneum entered by giving a slight jerk to the syringe. As the point enters the peritoneal cavity the resistance ceases suddenly and the tip of the needle becomes freely movable among the intestines. Care should be taken to avoid puncturing any of the organs by a hasty movement of the tip or by passing the needle too deeply. About five to ten c.c. of the serum or blood should now be slowly injected. The fluid should always be warmed just previous to the injection by placing the test tube containing it in a vessel of hot water at about 45° C., if not, the animal may die of shock.

The puncture made by the needle may be closed by a drop of flexible collodion and no further dressing is needed. The injection should be repeated at intervals of from three to five days. The animal should be watched carefully, and if it does not feed well or if it is losing weight, the injections should be discontinued for several days. From six to eight injections are usually sufficient to produce a serum of high precipitating power. Much smaller quantities of blood injected intravenously will give a strong antiserum; often two or three injections of two c.c. at intervals of a week will produce a satisfactory serum.

One or two weeks after the last injection, a few drops of blood should be drawn from the ear and allowed to clot in a small test tube or Wright capsule (Fig. 178, page 674). A few drops of the clear serum should be allowed to fall into a small test tube containing a 1 to 1,000 solution of dried blood in 0.85 per cent. sodium chloride. If a cloud is produced in one to two minutes, the serum may be considered to be satisfactory and the animal may be bled. If a cloud is not produced in that time the animal should be rejected.

### OBTAINING THE BLOOD

The rabbit should be tied down to an operating board which is covered with a large rubber hot-water bag,[1] previously filled with water at a temperature of about 54° C. If much bleeding is to be done without assistants, the animal may be conveniently held in a zinc box with a sliding top, the walls of which can be filled with hot water. The animal's head protrudes from the box; and if during the bleeding an electric light bulb be held under the ear, the veins will dilate and a very free flow of blood will be obtained. The external surface of the ear near the posterior border having previously been clipped or shaved, and cleansed, the ear is kneaded until the vessels become prominent; and the posterior auricular vein is incised with a sharp scalpel or fine-pointed scissors, the cut extending obliquely across the vessel, but not severing it. The blood, which flows freely from the cut under these conditions, is collected in small wide mouthed bottles holding about thirty c.c. Five c.c. of the blood is placed in each, and the bottles are laid on their sides until coagulation takes place, the mouth being closed by a sterile cork. When the coagulum has formed the bottles are placed vertically and left at room temperature for some hours, and then placed in a refrigerator for forty-eight hours. The exuded serum should be poured off into small test tubes, which should be plugged with sterile cotton and sealed with paraffin, or the tube may be drawn to a point and closed in the flame of a blowpipe. If positively sterile, the serum may be kept in the dark at room temperature, but if there is any doubt as to its freedom from organisms, it had much better be stored in the refrigerator. Instead of wide mouth bottles, the Wright capsules may be used.

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[1] We owe this method of obtaining large quantities of blood from the ear vein to *Wadsworth:* Proc. of the N. Y. Path. Soc., 1903, N. S., vol. iii, p. 112.

Uhlenhuth prefers to anesthetize the animal, open the thorax, incise the ventricle with scissors, and collect the blood by a sterile 50 c.c. pipette from the pleural cavity.

A still more elaborate method of bleeding the animal is to chloroform it, tie a small glass cannula into the carotid artery, and by a connecting bit of rubber tubing draw off the blood into sterile test tubes. In this way all possibility of contamination is avoided. The test tubes are plugged and laid in a sloping frame until the blood has clotted, and the serum is poured off at the end of twenty-four hours. If left too long in contact with the clot, the red corpuscles will be laked and the serum will be colored with hæmoglobin. It is important that the animals be starved for twenty-four hours before the bleeding is done, as otherwise the blood may contain considerable quantities of fat. If the serum is cloudy, it must be filtered with aseptic precautions through a small Berkefeld filter.

## APPLYING THE PRECIPITIN TEST

Before applying the precipitin test to a suspected stain, the presence of blood should be determined, either by the formation of Teichmann's crystals or by the spectroscope. Chemical reactions with guaiac and benzidin have less medico-legal value as they are given by other substances.

**Obtaining a Solution of the Blood to be Tested.**—Fresh blood stains which have not been exposed to the action of the sun or to high temperatures often promptly dissolve in 0.85 per cent. sodium chloride solution.[1] If not, distilled water may be used, an equal bulk of 1.7 per cent. salt being added to bring the solution up to 0.85 per cent. Old stains require hours for solution. If possible the fragments of blood should be removed from the cloth or instrument on which they are found so as to obtain as pure a solution of the blood as is possible without contaminations. The solution should be tested with litmus and neutralized by sodium carbonate or tartaric acid as may be necessary. The blood solution must now be filtered through filter paper or a miniature Berkefeld filter to render it perfectly clear. Schleicher and Schüll's No. 597 is a good paper for this purpose. If the fluid is still turbid after the filtration, prolonged sedimentation on a high speed centrifuge may produce a clear solution. It is important that the blood to be tested should not exceed a concentration of 1 part in 1,000. Such a solution forms a layer of foam when shaken, and on heating and adding 1 drop of a 25 per cent. solution of nitric acid, a slight opalescence only will appear.

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[1] Merck's guaranteed reagent sodium chloride should be used, as commercial salt gives somewhat varying results.

**Obtaining the Reaction.**—The reaction is carried out in small test tubes about 10 cm. long and 8 or 9 mm. in diameter. These must be absolutely clean and sterile. The cleansing is easily done by allowing the test tubes to soak for some time in a mixture of one volume of concentrated hydrochloric acid and four volumes of 97 per cent. alcohol. In fact, it is convenient to store the tubes in this mixture. They are then washed in distilled water, drained, and sterilized.

A series of such tubes is set up in a rack, numbered, and filled as follows:

1 and 2. One c.c. each of a 1 to 1,000 solution of the blood stain to be tested.

3. One c.c. of a 1 to 1,000 solution of known fresh normal human blood in 0.85 per cent. salt solution.

4. One c.c. of a 1 to 1,000 solution of dried human blood in 0.85 per cent. salt solution.

5, 6, and 7. One c.c. each of a 1 to 1,000 solution of blood from some of the domestic animals, as, for example, chicken, dog, and horse.

8. One c.c. of a sterile 0.85 per cent. salt solution.

9. One c.c. of an extract of the cloth, wood, paper, or other substance upon which the suspected stain was found. The reason for this is that cloth, wood, or paper may contain substances which would cause precipitation in rabbit antiserum.

To each of these tubes, with the exception of No. 2, is added with an accurately graduated pipette, 0.1 c.c. of the antiserum, the potency of which has previously been tested, the fluid being allowed to run down the wall of the slightly inclined tube. To tube No. 2 should be added 0.1 c.c. of normal rabbit serum.

In about two minutes a delicate clouding will appear at the bottom of the tubes containing human blood, with the exception of No. 2. In all others no clouding will appear.

**Sources of Error.**—The substances on which the blood has been deposited may interfere with or entirely prevent the reaction. Blood stains on linen, cloth, or paper, usually give prompt results. When the blood has been dried on leather the conditions are less favorable, as the tannin or the acid present under these conditions may produce spontaneous precipitates without the addition of antiserum. Stains from wall-paper often give spurious reactions depending probably upon chemicals used in the coloring. Ewing was unable to obtain satisfactory results from human blood stains on starched cuffs. The writer, however, in a medico-legal case, found no such difficulty.

Blood mixed with earth usually contains lime, and this must be removed by a current of $CO_2$ before the application of the test, but tests made under these conditions do not often succeed, especially if the blood has remained moist for a month or more. Mortar, lime, and fresh plaster completely destroy the precipitable substance of the blood. Strong acids and alkalies do the same. Blood dried on metal instruments [1] even after thirty years will give the reaction.

Inasmuch as any fluid which contains serum derived from the body may give this reaction, great care should be taken in the identification of blood stains from cloth which may have been contaminated either with nasal or bronchial secretions, which give a faint precipitation reaction, or with albuminous urine or diarrhœal discharges from the intestines, both of which may give the reaction when dried on clothing, though, as the writer has found, usually only a faint one. Vaginal, lochial, and seminal fluids also give precipitates.[2]

In investigating the handles of instruments, the possibility that they may have been contaminated with serum derived from blisters on the hands of persons using them should always be kept in mind. Another thing which must be considered is the possibility of confusing human and ape blood. As has been previously mentioned, the precipitates produced by human blood are most marked in the blood of the higher apes, such as the orang-outang, contamination with the blood of which need rarely be thought of. The more distant species of the monkey tribe give only faint precipitates. If the antiserum be diluted 1 to 1,000, and the conditions are favorable, it is unlikely that any confusion will arise.[1] Control tests should, however, always be made, if possible, with monkey blood, in order to determine the exact precipitating limits to monkey blood of the serum used in carrying out the tests.

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[1] *Graham-Smith* and *Sanger:* Journal of Hygiene, 1903, vol. iii, p. 269.

[2] *Biondi:* Viert. f. ger. Med., Bd. xxiii, Supplement-Heft, 1902, p. 1.

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[1] *Kister* u. *Wolff:* Zeit. f. Hygiene, 1902, Bd. xli, p. 410; *Ewing* and *Strauss:* Medical News, 1903, vol. lxxxiii, p. 871.

# PART II

# EXAMINATION OF THE GASTRIC CONTENTS

## I. GENERAL CONSIDERATIONS

THE gastric juice is the product of the secretory activity of the glands of the stomach wall. It is a thin, highly acid fluid, which contains but little solid matter and has a specific gravity of 1.001 to 1.010, and a freezing point of from —.38° to —.44° C.[1] On chemical analysis the gastric juice is found to be chiefly composed of dilute hydrochloric acid of a strength of from 0.1 to 0.3 per cent., a very small amount by weight of pepsin, a milk coagulating ferment (chymosin), together with a fat-splitting ferment (lipase), a little mucus, and some salts. The salts are chiefly chlorides and phosphates.

The ferments, the acid, and the mucus, seem to be secreted each by a set of highly specialized cells, among which the parietal cells probably furnish the acid while the chief cells furnish the ferments. The pepsin is secreted by the glands both of the fundus and pylorus, while the milk coagulating ferment and the lipase seem to be entirely derived from those of the fundus.[2] The ferments are not secreted as ferments, but in the form of inactive zymogens, which must first be split up to be active. The active agent in this splitting process is the free HCl of the gastric juice.

The fasting stomach usually contains a small amount of gastric juice, varying from 20 to 100 c.c.[3] Anything above this amount is to be looked upon as pathological. The total quantity secreted per day is not accurately known, but probably amounts to at least 2,000 c.c.

The **hydrochloric acid** of the gastric juice has a number of functions. In the first place, it is antiseptic and inhibits the growth of many, though not all, varieties of bacteria in the stomach. Secondly, it has the power of changing the inactive zymogens into the active ferments. This action is very rapid and requires only a few seconds. The hydrochloric acid also combines with the proteid constituent of the food to form acid albumins as a necessary preliminary to the action of the pepsin upon these substances. It also has the power of inverting cane-sugar to dextrose and lævulose. Finally, the acid gastric juice when it enters the duodenum acts as a powerful stimulant to incite the pancreatic secretion. The acid checks, as a rule very quickly, the action of the ferment of the saliva, though this is somewhat dependent upon the amount of mixture which has taken place between the saliva and the food. If this mixture is very thorough, the salivary ferment can still act for a considerable time after the food has been taken into the stomach.

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[1] *Roth* u. *Strauss:* Zeit. f. klin. Med., 1899, Bd. xxxvii, p. 144.

[2] *Glaessner:* Hofmeister's Beiträge, 1901, Bd. i, p. 24; *Volhard:* Zeit. f. klin. Med., 1901, Bd. xlii, p. 414.

[3] *Boas:* Magenkrankheiten, fifth edition. Many writers, however, consider any amount over 50 c.c. as pathological.

The **pepsin** has the power of altering the acid albumin formed by the action of the hydrochloric acid upon the proteid of the food into simpler compounds, the albumoses, which are easily absorbed by the mucous membrane of the stomach and intestines, while albumin can not be so absorbed. Peptone is not, as a rule, present in the stomach except in traces.

The amount of proteid digested is as the square root of the ferment present,[1] or expressed in another form, relative amounts of pepsin present in two fluids, one of which digests two grams of coagulated proteid in an hour and the other three grams, are not as 2 to 3, but as the square of these members—i.e., as 4 to 9.

The **milk coagulating ferment,** also called rennin, lab, or chymosin, has the power of coagulating the casein of the milk as a preliminary to its further digestion by pepsin. The rate of coagulation varies with the concentration of the ferment.

The **fat-splitting ferment** is capable of breaking up large quantities of fat when the latter is in finely divided form. The ferment exists in the form of a zymogen, which is activated by the hydrochloric acid of the gastric secretion, just as is the case with pepsin. The laws governing the splitting by the lipase are the same as those which regulate the action of pepsin—that is, the digestive products are as the square root of the amount of ferment present and as the square root of the time of digestion.

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[1] *J. Schütz:* Zeit. f. phys. Chem., 1900, Bd. xxx, p. 1.

## II. METHODS OF OBTAINING GASTRIC JUICE

### TEST MEALS

The gastric juice is not secreted continuously in any quantity, but normally only in response to some food stimulus. It has been assumed from the results of animal experimentation, chiefly by Pawlow[1] and his pupils, that the stimulation to secretion is almost wholly psychical and that neither the act of chewing nor the taste of the food exerts any great effect. It is probable that the psychic element plays a very important part in man in the stimulation and inhibition of the secretion of the gastric juice, though observations made on persons with gastric fistulæ have not furnished consistent results. A good many observers are inclined to think that the chief factor in the stimulation is the contact of food with the gastric mucosa, a belief in accord with the results obtained by forced feeding through the stomach tube, where the psychical element, if present, would certainly inhibit and not stimulate secretory activity. Others have found that the tasting or chewing of palatable forms of food[2] determines an active secretion. The entrance of the saliva into the stomach has also been considered a stimulant.

For clinical purposes we must rely upon the stimulus of food, using such forms as least interfere with the action of the indicators used in performing the analysis and do not contain such chemical substances as may be produced in the stomach by bacterial or secretory activity. The test meal is given fasting, and after a suitable time is removed from the stomach and examined.

**Ewald Test Meal.**—This test meal is most frequently used in the ordinary routine of stomach examinations. The meal consists of a roll or slice of bread without butter, and a glass of water or a cup of tea without milk or sugar; or, more accurately, about 50 grams of bread and 400 c.c. of fluid, though the exact amounts are of no very great importance. The patient should be instructed to chew the bread very thoroughly, for if coarse particles are swallowed they may plug the openings in the stomach tube and prevent the aspiration of the test meal.

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[1] Die Arbeit der Verdauungsdrüsen, Wiesbaden, 1898.

[2] See on this subject, *Hammarsten:* Lehrbuch d. Phys. Chemie, 1904, fifth edition, p. 298; also *Bulawinzew:* Wratch, 1903; abstract in Biochemisches Cent., 1903, Bd. i, p. 593 (Obtained secretion after psychic stimuli); *Schüle:* Deut. Arch. f. klin. Med., 1901, Bd. lxxi, p. 111. [Bibliography.] (Psychic action unimportant; chief action from tasting and chewing food and direct stimulation on gastric mucosa); *Meisl:* Wien. klin. Rundschau, 1904, p. 241.

The gastric contents are to be removed one hour later. Under these circumstances we expect to obtain from 30 to 50 c.c. of gastric contents, depending, however, a good deal upon the skill with which the tube is used. If the motor activity of the stomach is diminished or the organ is dilated, we will obtain a larger quantity of fluid. If, on the other hand, the activity is excessive, we will obtain little or no gastric contents.

**Riegel Test Meal.**—A second form of test meal, which is useful in determining the general digestive power of the stomach, but is not so valuable for chemical investigation, is that of Riegel. This consists of a plate of bouillon, about 200 grams of beefsteak, two slices of white bread, or 150 grams of finely mashed potatoes, and a glass of water. The contents of the stomach are to be removed at the end of from three to four hours.

The special value of this test meal is that it gives us the opportunity to judge of the length of time which the food remains in the stomach after a normal meal, and also to judge of the rate and the amount of digestion which has taken place, during that time, of the starches and of the proteids. The test meal of Ewald does not permit of this, as it is withdrawn before digestion has had an opportunity to advance very far.

If the stomach is found practically empty at the end of three hours, the motility of the organ may be assumed to be normal or increased, but no conclusions can be drawn as to the digestive power of the secretion. This may be low, and must be determined after an Ewald meal and chemical tests.

If the stomach still contains large quantities of undigested food at the end of four hours, the digestive power must be considered as low. The chief advantage of the Riegel test meal is that it is more palatable to the patient than the Ewald meal and presumably incites a more nearly normal gastric secretion than the latter. One of the difficulties of the method is that it is often impossible to prevent the clogging of the stomach tube by the particles of undigested food. The patient should therefore always be instructed to chew the food thoroughly.

**Sahli Test Meal.**—When employing the Ewald test meal it is impossible to decide accurately how much of the stomach contents removed is the fluid secreted by the gastric mucosa and how much the remnants of the fluid and food taken in. This is because such a meal is of inconstant composition and contains no substance which can be easily estimated in a quantitative manner. The meal when removed often contains coarse fragments derived from the imperfect mastication of the roll, and some of these masses are often left in the stomach because they can not be removed through a stomach tube of convenient caliber. A test meal of milk of a known fat content would permit the exact determination of the amount of the meal still remaining in the stomach by the simple process of estimating the amount of fat contained in a given fraction of the contents after evacuation through the tube, but the proteids of milk are so abundant that the coagula are very coarse and carry down most of the fat, while usually no free hydrochloric acid is present at the end of an hour, the whole of the acid having entered into combination.

Sahli[1] has therefore suggested the use of a soup containing a small amount of proteid and a moderate amount of fat in a state so finely divided that it will not separate during digestion, but the whole meal remain homogeneous. A fat determination made on a small fraction of the meal will permit of an estimation of the total amount ingested and the amount of the meal still remaining in the stomach. Fat, as is well known, is but slightly acted upon by the ferments of the gastric mucosa except when in a very fine emulsion, and the slight amount of splitting which does occur is within the limits of error of the methods used to estimate the amount present in the stomach contents after removal. There is also no absorption of fat from the stomach, so that any loss must be due to the expulsion of a portion of the meal through the pylorus, thus furnishing an index of the motility of the stomach.

A soup is prepared by mixing 25 grams of flour and 15 grams of butter in a pan and browning them over the fire. Three hundred and fifty c.c. of water are then slowly added and the whole allowed to boil for two minutes. The mixture is then suitably seasoned with salt and is ready for use. The fluid so prepared forms a palatable[2] emulsion from which the fat has but little tendency to separate, even when acted upon by the gastric secretions. The fat content of any portion is therefore quite constant, and, when the stomach contents are withdrawn, the amount of fat in a given quantity is easily determined, thus permitting an estimation of the residue of the test meal remaining in the stomach, also the amount which has passed through the pylorus, and the amount of gastric juice secreted by the stomach.

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[1] Berl. klin. Woch., 1902, Bd. xxxix, p. 349; *Seiler:* Deut. Arch. f. klin. Med., 1901, Bd. lxxi, p. 271.

[2] This palatability is important, as food of a pleasant flavor affords a much more nearly normal stimulus to the gastric secretion than a roll and water.

The patient receives either 300 or 500 cubic centimeters of the soup after a preliminary lavage to remove food particles remaining from other meals. The larger amount is to be given to those with well-marked motility, for otherwise no contents may be obtained at the end of an hour. The soup is best eaten with a spoon, so that none of the solid particles may be left at the bottom of the bowl. Fifty c.c. are to be reserved as a control for the determination of the fat content.

The contents of the stomach are to be expressed at the end of an hour and the stomach washed with 300 c.c. of water and the washings collected separately. The acidity of the undiluted contents and that of the washings are to be determined separately and the total secretion determined by Mathieu's formula (p. 271). The determination of the acidity should be made as soon as possible after the expression of the test meal, as the fat-splitting ferment of the gastric juice may break up the butter fat and set free butyric acid, which would add to the total acidity.

If it is not possible to carry out the titrations immediately, the action of the ferment may be checked by boiling the stomach contents for a few seconds. If the HCl content of the gastric juice is found to be low, it is well to remove any organic acid which may be present, by shaking 5 c.c. of the juice with 20 c.c. of ether and then titrating the aqueous portion for HCl. The other tests for combined acid and for lactic acid can be carried out on the undiluted stomach contents as with the Ewald test meal. The fat content of the gastric juice, the washings, and the soup are then determined by the Babcock method, as is given under the head of Milk Testing.

Recent studies [1] of this procedure have shown that there is a tendency for the fat to separate from the fluid unless the stomach is actively motile. This at once vitiates any computation based upon the fat analysis of the different portions of the fluid in cases where the stomach is dilated or the pylorus obstructed. Further investigation is required to determine the exact limits of applicability of a method theoretically very valuable.

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[1] *Koziczkowsky:* Deut. med. Woch., 1902, p. 462; *Bönniger:* Münch. med. Woch., 1902, p. 1786.

All of these test meals are given preferably in the morning before any other food has been taken. In general it is better to do this than to remove any food possibly present by washing the stomach, for the action of washing out the stomach interferes with the normal course of secretion of the gastric juice. In cases of greatly dilated stomachs, it may, however, be necessary to wash out the stomach as a preliminary to the test meal. Under ordinary conditions this is never required.

One reason for giving the test meals at a fixed hour is that the secretion of gastric juice varies somewhat, depending upon the time of day. It has been found experimentally that a larger quantity of free hydrochloric acid is secreted at noon than in the early morning; so that it is better to follow the precedent which has been long in use and give these meals early, before taking other food.

## SPECIAL TEST MEALS

**Boas Test Meal.**—This meal consists of about 500 c.c. of oatmeal soup prepared by boiling a tablespoonful of oatmeal (Knorr's "Hafermehl" preferably) with 1,000 c.c. of water until the bulk is reduced one-half. The contents of the stomach may be removed one hour after the meal though occasionally it is preferable to allow the gastric secretion to act for two hours or longer, as larger amounts of lactic acid are formed. This is only possible when the motility of the stomach is low, and under such conditions the meal may be given in the evening and removed the following morning. The especial purpose for which this meal was devised is to obtain stomach contents to which no lactic acid has been added from the food, for bread contains a small quantity of lactic acid, and this is not present in the oatmeal soup. One difficulty in connection with this test meal is the fact that it is sometimes impossible to obtain any quantity of gastric contents even at the end of an hour. If the stomach possesses normal digestive powers the test meal may have been entirely digested and passed on into the intestine. We can then expect a considerable quantity of gastric contents after this meal only when there is either dilatation of the stomach or some obstruction at the pylorus, with lowered motility.

**Test Meal to Determine the Motility of the Stomach.**—In patients suffering from dilation of the stomach or from obstruction of the pylorus, it is sometimes of importance to know whether food is retained for a long time or not. The simplest means of obtaining information on this point is to remove all food from the stomach by thorough washing and then to feed the patient with something which can be easily recognized if not thoroughly digested. Raisins or fruit cake containing currants are the most convenient form of food to use, and a quantity of the indigestible portions of these fruits may often be found in a dilated stomach days after they were ingested.

## OBTAINING GASTRIC CONTENTS

Some patients can vomit the gastric contents at will, but this is rarely possible in those who are not trained. It is usually necessary to remove the contents through a stomach tube. The tube is of soft rubber, about 75 cm. long, with a lumen some 6 to 7 mm. in diameter. The lower end may be open, or closed and rounded as preferred, the closed form being more difficult to clean. There should be two oval lateral openings on opposite sides.

The tube is moistened with warm water before use. The patient should sit up in a chair or in bed, and a towel or rubber sheet is wrapped about the neck to prevent soiling of the clothes by the saliva, which is usually very freely excreted, or by the vomitus which may be brought up while the tube is being passed. False teeth should be removed. The head of the patient should be tipped slightly forward and the mouth slightly opened. The tongue should never be pressed down by a depressor or the finger. The tube is passed gently back over the tongue until its tip strikes the posterior wall of the pharynx, when it turns downward of itself and easily passes into the stomach. The patient can often materially aid the passage of the tube by swallowing while it is being passed. In the case of a very nervous patient it may be necessary to apply cocaine to the pharynx in order to diminish its sensitiveness.

There is not the slightest danger of the tube entering the larynx, though the patient often struggles and becomes somewhat cyanotic. As soon as the tube is in position it will be perfectly easy for the patient to breathe, and that such is the fact should be explained before passing the tube. Occasionally a nervous person will find it very difficult to breathe or will choke or vomit while the tube is being passed. In such cases it is better to immediately withdraw the tube, explain the process carefully to the patient, and then make another attempt. If this fails, it is best to wait until another day and not alarm a nervous person by repeated attempts to pass the tube.

After the tube is in position and it is desired to wash out the stomach, a funnel is attached by another short piece of tubing and a glass connecting tube. About 500 c.c. of water are poured into the funnel and the latter elevated to about the level of the patient's mouth or a little above. The water will flow in quickly if the tube has been correctly passed to a distance of about 40 cm. from the incisor teeth, and if the funnel is lowered quickly the water will again siphon out into a basin, which should be near by. This procedure may be repeated as often as it is necessary to remove all of the mucus or small food particles from the stomach.

In case the siphonage does not take place immediately, it is well to push the tube a little farther into the stomach or to pull it out slightly. If this does not suffice, the patient should be asked to cough or bear down, and thereby start the siphonage. If this does not succeed, more water may be poured through the tube until the patient complains of a sense of fullness in the stomach. After this, if the funnel is lowered, the water will usually quickly siphon back, especially if the patient has aided the expression of the fluid by voluntarily compressing the abdominal muscles as in the act of vomiting.

If it is desired to obtain the stomach contents merely, without washing out the stomach, the tube should be passed as above, taking especial care that none of the saliva, which is usually freely secreted, is swallowed or runs down the tube into the beaker which is to receive the stomach contents. The best means of preventing the admixture of saliva and the gastric juice is to wrap a towel about the peripheral end of the tube, which will absorb the saliva as it flows along the tube from the mouth.

When the tube has been passed the normal distance of 40 cm. the patient should be asked to cough or make an effort to vomit, or to contract the abdominal muscles as if at stool, when usually siphonage will be established and the contents of the stomach will be expressed through the tube into the beaker intended to receive it.

If, however, the patient can not voluntarily express the contents of the stomach, it will be necessary to resort to aspiration. This can be accomplished either by the operator sucking gently on the end of the tube, or by attaching a small Politzer bag to the end of the tube by a glass connector after the bulb has been compressed, and then allowing the bulb to expand. The slight vacuum thus produced in the tube will usually start the removal of the contents of the stomach. Tubes are made which possess a small bag without valves at their upper end. (Fig. 69.) If the bag is squeezed and the peripheral end of the tube is closed by the finger and the bag again allowed to expand, a partial vacuum will be produced and siphonage of the stomach contents started.

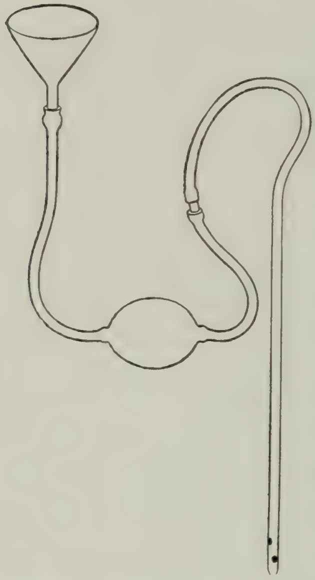

Fig. 69.—Stomach Tube with Bulb for Extraction of the Gastric Contents or Inflation of the Stomach.

When withdrawing the tube the patient should be asked to cough, to drive any remnants of gastric juice into the lower end of the tube. As this occurs the tube should be compressed by the finger and rapidly but gently extracted, keeping up the compression until the contents can be allowed to run into the beaker containing the portion previously removed.

**Contraindications** to passing the tube are uncompensated heart disease of any kind, either muscular or valvular, aneurisms, advanced pulmonary tuberculosis, apoplexy, or recent and very severe hæmorrhage from the stomach, especially in the case of ulcer or carcinoma of that organ. Menstruating[1] and pregnant women should not be examined unless the diagnostic or therapeutic results expected are of great importance. Ulcer of the stomach is not an absolute contraindication, however, if great gentleness is used in passing the tube and the stomach is not too much distended by fluid, but the tube should be used only for important diagnostic or therapeutic purposes. It is safer to treat a suspected case as ulcer and avoid the passage of the tube.

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[1] *Elsner:* Arch. f. Verdauungskrankheiten, 1899, Bd. v, p. 467. Results of examinations of gastric contents during menstruation are not constant, and often vary considerably from those obtained at other times. The nervous condition at this time probably plays an important part in the phenomenon.

## III. MACROSCOPICAL EXAMINATION

A great deal can be learned from the mere inspection of stomach washings after a test meal. In cases of diminished motility with dilatation or pyloric stenosis, a diagnosis can practically be made from the results of examination of the washings, which under these conditions contain food derived from previous meals.

The amount of bread contained in the washings after an Ewald test meal furnishes a clue to the digestive power of the stomach and also to its motility. The washings may contain no food in cases of hypermotility such as may be occasionally seen in connection with total absence of secretion of hydrochloric acid. In severe cases of chronic gastritis without increased motility the test meal will not be digested at all and the abundant mucus present will furnish a clue to the nature of the process. The increased mucus is easily noticed by stirring the contents or by pouring the washings from one beaker to another. Small quantities of mucus are found in the stomach under normal conditions.

The determination of the presence of pus usually requires a microscopical examination for the identification of the leucocytes present, though occasionally small masses may be found in the stomach contents derived from the rupture of abscesses on the walls of the stomach or by perforation from one of the neighboring organs, which can be identified macroscopically.

**Color.**—The presence of considerable quantities of blood is evident from the color. This is bright red in case of a fresh hæmorrhage from the œsophagus or a gastric ulcer, or of blood derived from the nose or throat of the patient. When the blood is thoroughly mixed with gastric contents and in the form of hæmatin or the so-called coffee-ground matter, its recognition is not so easy and suitable tests should be applied. (See pages 11, 18, and 274.)

Bile is easily identified by its green tint, though occasionally sufficient bilirubin may be present to color the stomach contents yellow. As the pigment is usually already oxidized to biliverdin it is often difficult to carry out the usual color tests (see Urine); but, when the question is of importance, the presence of bile may be shown by isolating the bile acids and testing them or by demonstrating considerable amounts of cholesterin. (See, however, p. 276.)

**Odor.**—Pure gastric juice or normal stomach contents after a test meal is practically odorless or usually has only a slightly acid smell. The admixture of intestinal contents may usually be recognized by the odor, but in case of doubt the suspected fluid may be distilled with a little phosphoric acid and tested for indol or phenol, as described in the chapter on Urine.

The washings from a dilated stomach are often sour-smelling, due chiefly to butyric, acetic, or valerianic acids. The broken-down tissue from an ulcerating carcinoma gives the washing a strong odor of putrefying flesh.

A very offensive odor may be obtained in washings from a normal stomach when the products of putrefaction are derived from abscesses or necrotic new growths in the œsophagus or pharynx, or from discharges from abscesses of the frontal or other sinuses.

**Amount.**—The quantity of fluid obtained at the end of an hour after an Ewald test meal is from 20 to 50 c.c. If larger amounts, up to 200 or 300 c.c., are obtained, it is an evidence of either diminished motility or hypersecretion. Large quantities, of 500 c.c. to 3 or 4 liters, indicate dilatation of the stomach and usually either benign or malignant stenosis of the pylorus.

The formation of three distinct layers in the washings is not infrequently observed when the stomach is greatly dilated. The upper portion consists of coarse fragments of undigested food, derived from the test meal; below this is a clear layer of fluid; while at the bottom may be observed a layer of fine particles of more or less digested food. In this lower layer will be found fruit skins, and other evidences of prolonged stasis.

## DETERMINATION OF THE TOTAL AMOUNT OF GASTRIC JUICE SECRETED

In order to determine the total amount of gastric juice secreted, a somewhat complicated procedure is necessary. It is evident that measurement of the quantity removed does not give us the total secretion, as it is impossible to obtain the last few c.c. from the stomach. The method of Mathieu and Rémond[1] is one of the best for this purpose.

As much as possible of the gastric juice is removed from the stomach, measured, and set aside. A measured quantity (usually 300 c.c.) of water is then poured into the stomach through the tube and thoroughly mixed with the contents remaining in the organ by moving the funnel up and down or by intermittent pressure on the stomach through the abdominal walls. As much as possible of the mixture of water and gastric juice is then removed and collected in a separate vessel. If $b$ is the quantity of gastric juice obtained by direct expression before the addition of water, $a$ the total acidity of this undiluted juice as determined by phenolphthalein, $c$ the acidity of the diluted gastric juice, and $q$ the amount of water added to the stomach, then the acidities $a$ and $c$ are inversely as the quantity of water used, since the greater the amount of the wash-water, the less the total acidity of the fluid.

This may be expressed by the following formula:

$$a : c = q + x : x$$

From which we derive $x = \frac{cq}{a - c}$

The amount of gastric juice originally present in the stomach is therefore $b + \frac{cq}{a - c}$

This formula assumes that acid is present in the stomach contents. In diseases where no free acid is present, sufficient acid may be given by the tube to cause an acid reaction in the stomach or the method devised by Strauss[2] employed. This depends upon the relations between the specific gravity of the diluted and undiluted gastric juice. This must be very carefully determined by means of a pycnometer bottle or Westphal balance. If then S is the specific gravity of the undiluted contents and G the specific gravity of the diluted contents, V the amount of juice first removed and $a$ the amount of water added, the total secretion is—

$$\frac{\mathrm{V\,S} + (a - \mathrm{V})\,\mathrm{G} - a}{\mathrm{S} - \mathrm{G}}$$

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[1] Soc. de Biol., 1890, p. 591; also Arch. f. Verdauungskrankheiten, 1896, Bd. i, p. 345.

[2] Therap. Monats., 1895, p. 125.

This formula can be simplified, as suggested by Reichmann,[1] by determining only the amount of secretion remaining in the stomach, not the total. The formula is then—

$$X = \frac{a\,(G-1)}{S-G}$$

Then X plus the amount of juice first removed is the total secretion.

## IV. MICROSCOPICAL EXAMINATION

Usually the microscopical examination of the gastric juice is carried out on test meals removed by the stomach-tube, but occasionally it is of importance to examine specimens of vomitus.

The secretions from the fasting stomach consist chiefly of gastric juice mixed with small quantities of mucus and saliva. The salivary admixture can be easily shown by the presence of the large flat epithelial cells from the buccal cavity and the shrunken leucocytes and cell nuclei known as salivary corpuscles.

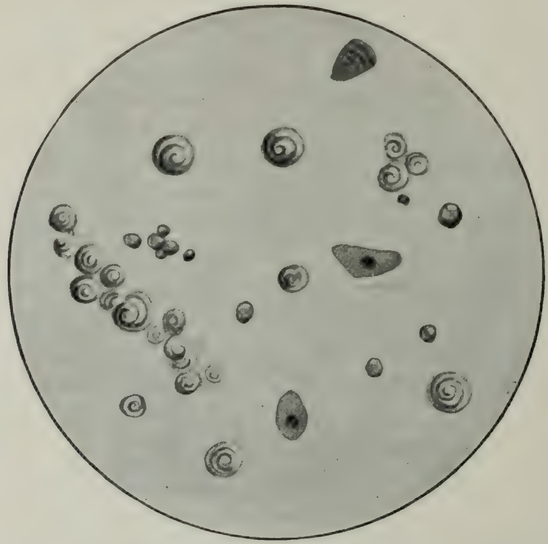

Fig. 70.—Normal Stomach Contents, Showing Snail-like Masses of Mucus, a Few Flat Epithelial Cells, and an Occasional Starch Granule.

As a rule the gastric juice is never entirely free from small remnants of food, even though nothing has been taken for twelve or fifteen hours. Elastic tissue and muscle fibers are usually present, together with fat drops and fatty acid crystals, starch, and fragments of plants.

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[1] Deut. med. Woch., 1895, Lit. Beilage, No. 12, p. 79.

Small masses of mucus can nearly always be recognized in the acid gastric secretions, and occasionally assume a peculiar snail-like, spiral form (Fig. 70). They are probably formed from nasal or bronchial or gastric mucus by the action of the hydrochloric acid of the stomach. A few bacilli, molds,[1] and yeast cells are nearly always present. They are of no diagnostic importance.

The microscopical examination of the gastric juice after a test meal shows, besides the elements mentioned above, numerous substances derived from the roll or other material given to incite the gastric secretion. With the Ewald meal little is to be seen except numerous starch granules and vegetable cells. Some of the starch granules are well preserved; others have been destroyed in the process of cooking, the cellulose membrane of the starch cell having been ruptured by this process. These remnants of starch cells may be identified by treatment with iodine, when they assume a blue color. After the Riegel test meal a number of more or less digested muscle fibers may be found, in addition to starch and vegetable cells.

If there is any interference with the motility of the stomach, or if the lumen of the pylorus is blocked by a new growth, one commonly finds remains of food which have been introduced into the stomach at previous meals, often twenty-four to forty-eight hours before. These remnants are chiefly of the more indigestible sorts of food, such as vegetable fibers and cells, and especially the skins from fruits, such as prunes, raisins, etc.

Fat appears in the form of spherical, highly refractile globules, which can be easily recognized by their solubility in benzine or ether, or by their micro-chemical reactions with Sudan III, scharlach R., or osmic acid. The Sudan III and scharlach R. color the fat an orange red, while osmic acid blackens it. A drop of a saturated alcoholic solution of these dyes should be allowed to run in under the cover glass and mix with the stomach contents. Osmic acid should be used in a 1 per cent. aqueous solution.

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[1] *Kellog:* Medical News, 1900, vol. lxxvii, p. 88.

Fatty acids assume the form of long needles, which also react to the fat stains. These may be single or arranged in bundles. Large quantities of these substances are seen chiefly in dilatation of the stomach. Neutral fat is not often seen in normal stomach contents after an Ewald test meal.

Red blood corpuscles in small numbers are frequently found after expression of the stomach contents, if the latter are examined promptly. Their appearance is due to small hæmorrhages produced by the action of the tube on the mucous membrane of the pharynx or the stomach. If normal amounts of hydrochloric acid are present the red cells are rapidly destroyed and the hæmoglobin altered into insoluble brown masses of hæmatin. If the stomach contents are neutral, however, they may remain unchanged for a considerable time.

The best method of testing for blood in the stomach contents is that of Weber.[1] A small portion of the "coffee-ground matter" is placed in a test tube and several drops of strong acetic acid are added and about one-third of its bulk of ether is poured into the tube and the whole is thoroughly shaken. After standing a few minutes the ether separates as a clear layer in the upper portion of the mixture and can be pipetted off. If the ether does not clear, a few drops of alcohol will assist in the process. The brownish solution is then examined with a direct vision spectroscope for the absorption spectrum of acid hæmatin. As a rule, only the band in the red is visible.

In many cases the hæmochromogen spectrum may be also obtained by treating the mixture with strong alkali, pyridin, and ammonium sulphide; but for small quantities of blood Weber's method is preferable.

The blood pigment can also be identified, according to Weber, by adding ten drops of a freshly prepared solution of resin of guaiac and twenty to thirty drops of ozonized turpentine to the ethereal extract of the stomach contents. If blood is present the ether assumes a purple color which can be extracted by shaking up with chloroform and water.

Instead of using tincture of guaiac to produce the color reaction an extract of Barbados aloin (see page 12) in dilute alcohol may be employed. The reaction is slightly more sensitive than with guaiac and the presence of free hydrochloric acid does not interfere with the color production, as is often the case when the latter substance is used.

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[1] Berl. klin. Woch., 1893, p. 441.

Teichmann's test for blood, preferably with Strzyzowski's modification (see page 18), may be used when blood is present in macroscopic amounts.

In persons with marked dilatation or carcinoma of the stomach, microscopical examination will reveal large numbers of yeasts, sarcinæ, and occasionally, especially in carcinoma, large rod-like bacteria, known as Boas-Oppler bacilli. The first two forms mentioned are not diagnostic of any special condition, as they are present in almost all stomach contents and only in increased numbers when there is abnormal stagnation of the gastric juice.

The Boas-Oppler bacilli frequently form long jointed chains. The fresh preparation should have some Gram's solution added to it to distinguish the Boas-Oppler bacilli from a mouth bacillus, the Bacillus maximus buccalis (Leptothrix buccalis). The latter will be stained blue by the iodine, while the Boas-Oppler is stained brown. The bacillus has been cultivated, but with some difficulty, so that the diagnosis must rest upon the morphology. The presence of the bacillus is confirmatory evidence merely of the presence of a new growth and possesses about the same value as the presence of lactic acid, as it is quite constantly found in the same circumstances as the acid, that is, in from 75 to 85 per cent. of the cases of carcinoma, and rarely in dilated stomachs or in benign obstruction of the pylorus.

Occasionally infusoria may be present in the stomach, especially the flagellate group;[1] but they are of no diagnostic importance.

Small fragments of the gastric mucous membrane are frequently found in vomitus or expressed stomach contents. They may be derived from a normal mucosa or from a variety of conditions. Einhorn thinks them especially abundant in cases of erosion of the stomach. Such fragments should be collected, hardened, embedded, and sectioned, in order to obtain satisfactory results from their examination. These fragments do not, however, always furnish valuable points in diagnosis or prognosis, for particles showing an atrophic mucous membrane may be obtained from persons without marked lesions of the stomach, at least so far as can be judged from symptoms and chemical examination of the washings. It is possible, therefore, that local alterations in the gastric mucosa may exist and from these areas a fragment may be carried away by the action of the tube or the disturbance due to expression.[1] So, too, an advanced atrophy of the mucosa may exist, as shown by examination of fragments and chemical tests, and yet the patient may have no symptoms referable to that condition.

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[1] *Cohnheim:* Ueber Infusorien im Magen und im Darmkanal des Menschen und ihre klin. Bedeutung. Deut. med. Woch., 1903, p. 206.

Mitoses are occasionally found in the cells of the glands, which do not indicate a malignant growth but only a rapid proliferation of the glandular epithelium.

Fragments of carcinoma are occasionally found in the stomach washings and a diagnosis may be made from suitable stained sections if a growth of epithelial cells can be demonstrated between the tubules or in solid masses of cells with large nuclei. Very often, however, the fragments which fall from the surface of a growth of this type are so necrotic that a definite diagnosis can not be made.

Crystals of cholesterin and leucin are occasionally seen even though there is no regurgitation of bile. Tyrosin and calcium oxalate have been noted. Crystals of ammonium magnesium phosphate have been found in alkaline gastric juice.

## V. CHEMICAL EXAMINATION

The amount of gastric juice which may be obtained one hour after the administration of the Ewald test meal is from 20 to 50 c.c. Larger quantities indicate either increased secretion of the gastric juice or decreased motility of the stomach or an obstruction at the pylorus. The continuous finding of very small quantities indicates an extremely active peristalsis of the stomach or a pyloric incontinence. For clinical purposes we determine the amount of hydrochloric acid which is free in the gastric contents after a test meal, the amount which is united or combined with the food, and the amount of organic acids and acid salts which is present. We also determine the presence and activity of the pepsin and of the milk-coagulating and fat-splitting ferments. The most important of all of these for clinical purposes is the examination for the presence of free hydrochloric acid.

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[1] *Einhorn:* Deut. med. Woch., 1903, p. 776.

## FREE HYDROCHLORIC ACID

### QUALITATIVE TESTS

**Congo or Amido-Benzol Paper.**—The examination for free hydrochloric acid can be made in a qualitative manner by the use of paper colored by Congo red or dimethyl-amido-azobenzol. The Congo changes, under the action of the free hydrochloric acid, to a bright blue. This change can be observed when no more than 0.002 per cent. of free hydrochloric acid is present. The Congo paper does not react to phosphoric acid or to organic acids in the concentration in which they are usually present in the stomach contents. Rarely, in the presence of large quantities of lactic acid, a slight change to blue may be observed; but a very strong reaction does not occur under these circumstances.

Amido-benzol paper reacts only to free hydrochloric acid, turning an orange, if traces, or a red color if large amounts are present. Large amounts of organic acid may redden the paper, but practically they are not met with in the stomach contents.

Boas highly recommends tropæolin paper made by soaking filter paper in a saturated alcoholic solution of tropæolin 00 and drying. The paper is dipped into the gastric juice and then dried over a flame. If free hydrochloric acid is present the moistened spot assumes a lilac-blue color. Organic acids do not give this color.

**Günzburg's Method.**—A reagent[1] which is not subject to any of these sources of error is a mixture of phloroglucin, 2 grams; vanillin, 1 gram; alcohol, 30 cubic centimeters. The solution should be preserved in a dark bottle and not kept for more than a few months. The method is as follows:

One to two drops of this solution are placed in a small porcelain dish and dried by gentle heat. A drop of the gastric juice is then placed on the brown stain and the dish again gently heated until all the water has evaporated. If 0.005 per cent. of hydrochloric acid is present a faint red will be visible. If more, the reagent changes to a beautiful carmine red, which is unaltered on the addition of ether. This reagent reacts only to free mineral acid, which in the case of the gastric contents can be assumed to be hydrochloric acid. It does not react in any case to organic acids or to acid salts. Test papers containing this reagent can be used by dipping them into the gastric contents and gently warming them over a flame.

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[1] *Günzburg:* Cent. f. klin. Med., 1887, p. 737.

## COMBINED HYDROCHLORIC ACID

The entire amount of hydrochloric acid secreted by the action of the gastric mucous membrane can not be found as free acid, for the HCl set free soon after the test meal is taken into the stomach combines with the bread of the meal and forms a so-called combined hydrochloric acid. It has been found, for instance, that 50 gm. of bread will combine with 0.15 gm. of pure hydrochloric acid, and if the combination is tested no free hydrochloric acid will be found, the acid having entered into a loose combination with the proteid and other substances in the bread. The same phenomenon may be observed by adding hydrochloric acid to milk; 50 c.c. of milk will combine with 0.2 gm. of hydrochloric acid, and if the mixture is tested by the phloroglucin test no reaction for free hydrochloric acid will be obtained. An example of this "acid capacity" of milk is seen in nursing infants whose gastric contents contains no free HCl, all the acid having united with the milk.

The amount of combined hydrochloric acid is greater in the case of Riegel's test meal than with Ewald's, for the former contains a larger quantity of proteid. For about 35 minutes after taking the Ewald test meal all of the acid secreted by the stomach will be found in combination with the bread of the meal; at this time the amount of the combined acid will have risen to 0.15 gm. to the 50 gm. of bread taken in. From now on free hydrochloric acid makes its appearance and may rise as high as 0.2 per cent. under normal conditions. It is evident, therefore, that the determination of this combined hydrochloric acid is an important matter, for it is just this acid which has entered into combination with the proteid of the food which makes it possible for the pepsin to act upon the proteid. Of course, if free hydrochloric acid is present in considerable quantities, it is an evidence that there is an excess of acid over the amount required to combine with the food; but if no free hydrochloric acid be present, it is not proven that none has been secreted, so that we should also test for the combined hydrochloric acid in attempting to estimate the functional activity of the stomach.

A simple qualitative test for loosely combined acid is not known, the amount is obtained during the quantitative Töpfer test.

Titration with decinormal sodium hydrate, using litmus as an indicator, will, however, if no organic acids are present, give approximate results for the free acid plus the loosely combined, after an Ewald test meal.

## TOTAL ACIDITY

Besides the free hydrochloric acid and the loosely combined hydrochloric acid, there are also present in the stomach contents organic acids and acid salts, chiefly those containing an excess of phosphoric acid. The organic acids and the acid salts, as a rule, have no digestive power.

The organic acids, when present in large quantities, replace the hydrochloric acid to a certain extent in the formation of acid albumin for the digestion of the proteids.

## QUANTITATIVE DETERMINATION OF FREE HYDROCHLORIC ACID

Boas[1] uses Congo red as an indicator in titrating for free hydrochloric acid. Five c.c. of a 1 per cent. aqueous solution of the indicator are mixed with an equal quantity of the unfiltered gastric juice. Decinormal sodium hydrate solution is then added from the burette until the solution changes to a marked brick red. The number of cubic centimeters of sodium hydrate used enable us to compute the amount of free hydrochloric.

Riegel[2] recommends the use of Congo-red paper and phenolphthalein for the determination of free hydrochloric acid and the total acidity in the stomach contents.

Ten c.c. of the gastric juice are placed in a beaker and decinormal sodium hydrate solution is run in from a burette until a drop of the fluid removed by means of a small platinum loop no longer gives rise to a bright-blue color on Congo paper.

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[1] Magenkrankheiten, 1903, Leipzig.

[2] Erkrankungen des Magens, second edition, Wien, 1903, p. 107. The method was due originally to *Mörner:* Jahresbericht d. Thierchemie, Bd. xix, p. 253.

If the change of color is not clearly defined, that produced by the drop of gastric contents on the Congo paper should be compared with the color change due to an equal sized drop of distilled water. In case there is any doubt about the reaction a drop of the fluid may be removed and placed on an already dried layer of Günzburg's reagent. This is then heated over a flame and the presence or absence of red color noted.

A few drops of phenolphthalein are then added to the gastric juice and the titration with $^{N}/_{10}$ alkali carried further until a permanent rose color is produced. The end point of the first reaction gives the total free hydrochloric acid, the second, the total acidity.

### TÖPFER'S METHOD [1]

The method devised by Töpfer is the best for the clinical determination of both free and loosely combined acid, and total acidity. It is based upon the titration of the gastric contents with three distinct indicators, each of which reacts in a different manner. The titration is carried out with a decinormal solution of sodium hydrate.[2]

**Free Hydrochloric Acid.**—To determine the amount of free acid we use a 0.5 per cent. solution of dimethyl-amido-azobenzol in strong alcohol. Ten c.c. of the unfiltered gastric juice are measured out into a small beaker and a few drops of the indicator added, when, if hydrochloric acid is present, the yellow color of the indicator will immediately change to a bright red. If this change does not occur, no free hydrochloric acid is present and the method can not be used even for the determination of the loosely combined acid.

If, however, the change to red does take place, decinormal sodium hydrate is added from the burette until the color of the indicator has been changed from red to a bright yellow, and the number of cubic centimeters required is noted. The number of cubic centimeters used, multiplied by the equivalent factor of hydrochloric acid, 0.00365, will give the amount of free hydrochloric acid present in the gastric contents;[3] but it is far simpler for clinical purposes to record the results in terms of the number of cubic centimeters of decinormal solution used. In other words, if we have used 4 c.c. of decinormal solution to neutralize 10 c.c. of the gastric juice, we should call the free acid 40, and may write this as $\frac{40}{40}$ if preferred; for the amount of decinormal alkali required to neutralize 100 c.c. of such gastric juice would be 40 c.c., and as 40 c.c. may be considered as an average amount of free hydrochloric acid after an Ewald test meal, the amount may be expressed by the same nomenclature as that used in ophthalmology for recording the efficiency of the eye, using the denominator of the fraction as the normal, and the numerator as the amount found.

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[1] Zeit. f. phys. Chemie, 1894, Bd. xix, p. 104.

[2] See Appendix.

[3] A convenient mnemonic for this figure is the number of days in the year.

**The Total Acidity.**—The acidity due to all forms of free acids, the loosely combined acid, and the acid phosphates may be determined by titrating 10 c.c. of the gastric juice after the addition of a few drops of phenolphthalein solution. No change is noted after the indicator is added except the formation of a white cloud from the precipitation of the alcoholic solution by the water; but as each drop of the sodium hydrate solution falls from the burette and strikes the surface of the fluid in the beaker, a bright pink cloud appears at the point of contact, rapidly disappearing if the solution be stirred. As the end reaction approaches, the color caused by the alkali remains longer and is more difficult to remove by stirring, until finally a single drop will change the whole fluid to a bright pink which can not be removed by stirring. This is not the true end reaction; enough alkali should be added until finally a drop produces no change in the depth of color of the fluid.

The reason for this is that the acidity present is not neutralized until a deep pink is obtained. The acid salts present are the dihydrogen phosphates of the type $MH_2PO_4$ which are acid to phenolphthalein. The addition of sodium hydrate changes this to $Na_2HPO_4$. This salt, however, is alkaline to phenolphthalein, so that the transition from a colorless to a pink fluid takes place before all the $NaH_2PO_4$ is transferred to $Na_2HPO_4$. In order, therefore, to estimate the whole acidity due to the phosphoric acid, the addition of the alkali must be continued until the rose color is no longer deepened by a drop of the sodium hydrate. The end reaction is rendered sharper by saturating the solution to be tested with sodium chloride. This diminishes the dissociation of the $Na_2HPO_4$ into $NaH_2PO_4$ and NaOH, the latter giving rise to the alkaline reaction to phenolphthalein.[1]

**Loosely Combined Hydrochloric Acid.**—The hydrochloric acid in combination with the food of the test meal is determined by the Töpfer method of titrating 10 c.c. of the gastric juice with a decinormal sodium hydrate, using 1 per cent. aqueous solution of alizarin as an indicator. The end reaction with this indicator is difficult to obtain accurately, and the best method of making oneself familiar with the color is to carry out the following tests which Töpfer advises.

Two or three drops of the alizarin solution added to 5 c.c. of water will give a bright yellow. If now two or three drops of a 1 per cent. solution of disodium phosphate be added, a red or violet color will appear. This is not the end reaction, but the proper color may be obtained by adding two or three drops of the alizarin solution to a 1 per cent. solution of sodium carbonate. This will strike a deep violet with the alizarin solution and gives one the proper end reaction.

In other words, the sodium hydrate must be added from the burette until the yellow color has disappeared and the violet is no longer deepened by the addition of another drop of the sodium hydrate from the burette. This is best noted by placing the burette containing the solution over a piece of white paper and adding the drops carefully in the center of the fluid, and noting whether a darker color is produced at the place where the drop has fallen. When a drop of the sodium hydrate no longer produces a deeper colored cloud in the solution, the end reaction has been obtained, and the amount of sodium hydrate used should be read off from the burette.

**Calculation of Results.**—The results of the titrations are computed as follows:

(*a*) Dimethyl-amido-azobenzol reacts to free hydrochloric.

(*b*) Phenolphthalein reacts to free acid, the acid salts, and the loosely combined acid.

(*c*) Alizarin reacts to free acids and acid salts.

Thus, if one subtracts *c* from *b* it is evident that the amount of loosely combined acid will be obtained, for the only difference between the reagents *c* and *b* is in their reaction with the loosely combined acid. This loosely combined acid must be expressed in terms of hydrochloric acid, and preferably by the form given above, where the amount of acid is estimated as if 100 c.c. of the gastric juice were used.

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[1] For further details, see *Glaser:* Indicatoren der Acidimetrie und Alkalimetrie, 1901, Wiesbaden; and *Treadwell:* Analyt. Chemie, 1903, Bd. ii, Leipzig, p. 414.

The total acidity varies greatly. In health it may reach 50 or 80 c.c. of decinormal sodium hydrate. In disease it may be higher than these figures in hyperchlorhydria and very low in carcinoma. A neutral gastric juice is occasionally seen, but this usually indicates contamination with saliva, which is alkaline. The source of the error may be determined by adding a few drops of dilute HCl and some ferric chloride to the stomach contents, when the red ferric sulphocyanide will be produced if saliva be present. The loosely combined acid varies somewhat according to the nature of the test meal. It is higher with the Ewald than with the Sahli meal. In general the amount varies within the limits of 0.5 to 15 c.c. of decinormal soda.

The Töpfer method is the most useful for general clinical work, and the errors inherent in it do not in the least interfere with its practical value in roughly estimating the acid content of the gastric juice.[1]

The method is inaccurate when free hydrochloric acid is not present, as in this case the alizarin titration gives an incorrect result. The amido-benzol titration is also influenced by large quantities of organic acids; but, as a rule, if free hydrochloric acid is present, no amount of lactic or other organic acid is to be expected. If organic acids are present in large quantities, however, the end reaction is not accurate.

In case large quantities of organic acids are present it is best to remove them by shaking out with ether and then testing the remaining fluid. They are rarely present in quantity if free hydrochloric acid is found. The unfiltered gastric juice is used in all these tests because it has been found that if the food particles are removed they carry with them a certain amount of the free as well as the combined hydrochloric acid.

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[1] A very practical discussion of the subject may be found in a recent paper by *Benedict:* Medical News, 1904, vol. lxxxiv, p. 597.

### RÉSUMÉ OF THE RESULTS OF THE TÖPFER METHOD

The average amount of free hydrochloric acid found by the Töpfer method after a test meal in normal persons is quite variable. In health it averages between 30 and 40, but as high as 80 or 90 may be seen in disease. The total acidity is usually 50 to 80, but these figures vary not only with the time of day, the amount of acid being greater at noon than in the morning, but with different races and with people of different social conditions, the higher values being found among well-nourished people who use large quantities of meat. The lower values come from the inhabitants of the tenement districts. The amount of loosely combined acid varies greatly. For practical purposes the figures obtained can be combined with those of the free hydrochloric as representing the amount of useful acid secreted. The average is 10 to 15 c.c.

### METHOD OF MARTIUS AND LÜTTKE

#### (*Reissner's Modification*)

For very accurate determinations for scientific purposes the method devised by Martius and Lüttke,[1] as modified by Reissner,[2] may be employed. It is too complicated for the routine clinical determination of the free and combined acids of the stomach contents.

The solutions required are:

*a*. One-tenth normal sodium hydrate solution;[3]
*b*. One-tenth normal silver nitrate solution;
*c*. One-tenth normal ammonium sulphocyanide solution;
*d*. Litmus paper;
*e*. One per cent. alcoholic solution of phenolphthalein.

The stomach contents to be tested should be thoroughly shaken and filtered, as Reissner believes that it is impossible to obtain constant results with unfiltered juice owing to the food particles present. He recognizes, however, the fact pointed out by Martius and Lüttke that the total acidity in such cases is slightly lower than when the unfiltered juice is used.

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[1] Die Magensäure des Menschen, Stuttgart, 1898.

[2] Zeit. f. klin. Med., 1903, Bd. xlviii, p. 101.

[3] See Appendix.

*a.* Ten c.c. of the gastric contents are measured off and titrated with decinormal sodium hydrate until the mixture is neutral to litmus paper. This gives roughly the free plus the loosely combined hydrochloric acid. The free hydrochloric acid can also be determined in the same fluid by means of dimethyl-amido-azobenzol or Congo red, and the total acidity by means of phenolphthalein.

*b.* To another 10 c.c. of the gastric contents an amount of decinormal sodium hydrate is added which is equal to the amount required to neutralize litmus paper as determined by the previous titration. This converts the free $HCl$ into $NaCl$. The mixture is placed in a platinum crucible and evaporated to dryness on a water bath or over a flame, protecting the crucible from the direct action of the flame by an asbestos plate.[1] The residue is heated over a Bunsen burner as long as it burns with a luminous flame. Further heating should be avoided because of the loss of the chlorides from volatilization. The dried residue is moistened with distilled water and rubbed up with a thick glass rod, washed on to a filter, and the soluble salts extracted with about 100 c.c. of hot distilled water. The filtrate is collected in a measuring flask of 200 c.c. capacity, 20 c.c. of decinormal silver solution are added, and the mixture is filled up to 200 c.c. and filtered. One hundred c.c. of the filtrate are measured off and decinormal ammonium sulphocyanide solution is added from a burette until a permanent yellowish red color indicates the transfer of the excess of silver nitrate into sulphocyanide. This determination gives the hydrochloric acid plus the chlorides.

*c.* An exactly similar experiment is carried out with the addition of only 10 c.c. of silver nitrate solution and without previous neutralization by decinormal soda. The hydrochloric acid escapes on heating and the titration of the residue gives the chlorides.

*d.* The total chloride content of the stomach mixture is determined by adding 10 c.c. of the untreated and unfiltered stomach contents to 20 c.c. of the silver solution, diluting to 200 c.c., filtering from the precipitate of chloride of silver and titrating 100 c.c. of the filtrate with the sulphocyanide solution.

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[1] It is convenient to use a crucible of hemispherical form which can be set into a small wooden mold, so that the crucible will not be deformed when rubbing the charred contents.

The subtraction of the results obtained by *c* (chlorides) from that obtained by *b* (hydrochloric acid plus chlorides), gives the hydrochloric acid. The sum of the total chlorides, *d*, less the results of *a*, gives the volatile chlorine compounds.

## THE DIAGNOSTIC VALUE OF THE DETERMINATION OF HYDROCHLORIC ACID

For a practical guide in dispensary work for the approximate classification of cases, the mere determination of the presence of free hydrochloric acid with Congo or amido-benzol paper will answer all requirements. For the more intelligent treatment of a patient with stomach trouble the determination of the total amount of free hydrochloric and combined acid is useful, but the results must be interpreted with great caution and should be based upon not less than three examinations made at intervals of several days. If free acid is absent, tests for the ferments and lactic acid should be made. It has been found that the first test meal very often shows an exceedingly low value for free hydrochloric, for which no other interpretation can be made than that the patient's fear of the washing out of the stomach has inhibited the normal action of the stomach mucous membrane.

We may consider the following clinical conditions:

1. **Euchlorhydria,** in which there is present a normal amount of hydrochloric acid. We may exclude under these circumstances organic changes in the mucous membrane of the stomach, such as go with a chronic gastritis, and can refer the symptoms of this latter condition, if present, to a nervous dyspepsia. The presence of a carcinoma of the stomach is improbable, unless the new growth has arisen upon the base of an old ulcer—a very rare phenomenon. A normal amount of free hydrochloric may exist in connection with a marked atony of the muscular coat of the stomach and extensive dilatation of that organ.

2. **Hypochlorhydria.**—The amount of hydrochloric acid is low, under 0.1 per cent. The constant presence of such a condition may indicate a subacute or chronic gastritis, though the diminution may also be seen in connection with cases of ulcer of the stomach or duodenum or in early carcinoma, and in cases of dilatation of the stomach. The amount of acid is usually diminished in acute infectious diseases and generally in advanced tuberculous cachexias. Great care should be taken, in cases where an analysis

shows a reduction in the amount of free hydrochloric acid, to exclude an admixture of saliva with the stomach washings, such as may easily occur unless care is taken to wrap a towel about the upper end of the stomach tube while washing out the contents of the stomach.

3. **Hyperchlorhydria.**—There is an excess of acid above 0.2 per cent. This may be a symptom of an excessive physiological secretion of hydrochloric acid such as is occasionally met with in healthy people, or it may appear in the beginning of a gastritis or as a symptom of a neurosis, especially in the form of the so-called Reichmann's disease or continuous hypersecretion. The stomach may or may not be dilated under these conditions. Finally, a certain number of cases of ulcer of the stomach are connected with this hyperacidity. The presence of this hyperacidity with undoubted symptoms of carcinoma of the stomach suggests, but does not prove, that the carcinoma has developed upon the base of an old ulcer.

4. **Achlorhydria or Absence of Hydrochloric Acid.**—This is a most frequent symptom of an advanced chronic gastritis. It is seen also in connection with neuroses of the stomach, but the two conditions may be distinguished by the fact that in the neuroses the ferments are still present in normal or slightly diminished quantity, while in chronic gastritis the ferments disappear or are present only in traces when the acid is absent. The absence of free hydrochloric acid is also a corroborative evidence of carcinoma of the stomach, though it has long been known that this symptom is present in only a moderate number of cases. The acid is regularly absent in pernicious anæmia.

In very rare cases not only free hydrochloric acid is not present, but there is no acid found when the gastric juice is titrated with phenolphthalein. Such a condition may arise in atrophic gastritis without disturbance of motility. If the stomach were dilated lactic acid fermentation would be probable, with a total acidity of 30 to 40.

5. **Heterochylia** is a term used to designate a condition in which the acid varies greatly on successive examinations.[1] When, after a few examinations, a very variable amount of free acid is found, the condition is probably a neurosis or a moderately advanced

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[1] See *Hemmeter:* Diseases of the Stomach; also *Korn:* Arch. f. Verdauungskrankheiten, 1902, Bd. viii, p. 75.

gastritis; but occasionally in connection with carcinoma of the stomach there are noticed very great fluctuations in the amount of acid secreted. In secondary dyspepsia, such as follows pulmonary tuberculosis, cardiac disease, diabetes, nephritis, etc., regular variations in the amount of hydrochloric acid secreted have not been observed. These diseases may show either normal, diminished, or increased hydrochloric acid.

## ORGANIC ACIDS

In food there is always a small quantity of lactic acid derived either from milk, bread, or meat. After an Ewald test meal there may be traces of lactic acid derived from the roll, while the Riegel test meal always gives a stronger lactic acid reaction due to the presence of sarcolactic acid, a dextrorotatory isomer of the optically inactive lactic acid formed by fermentation. Other organic acids, such as butyric and acetic acids, are formed directly in the stomach by the fermentation of carbohydrates. Lactic acid is the only one of any great clinical importance. The qualitative reactions for lactic acid are easily applied and are of great value in diagnosis.

### QUALITATIVE TESTS FOR LACTIC ACID

**Uffelmann's test**[1] is one of the first devised, and is practically trustworthy in pure solutions of lactic acid; but, under conditions which frequently exist in stomach washings, the organic substances present interfere so greatly with the carrying out of this reaction that it is no longer considered of much value. It is given here merely because it is the classical reaction for lactic acid. Ten c.c of a 4 per cent. carbolic acid solution are mixed with 20 c.c. of water and a few drops of 10 per cent. ferric chloride. The mixture must always be freshly prepared. A few drops of $\frac{1}{10}$ per cent. lactic acid causes a beautiful canary yellow color to appear in the deep amethyst fluid.

The test is more reliable if the lactic acid is first extracted by shaking up the stomach contents with ether, and then allowing the ether to evaporate in an open dish and dissolving the residue in water and then applying the test as above. This removes many of the sources of error, especially those due to the phosphates, glucose, and albumose, all of which are contained in the gastric contents.

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[1] Deut. Arch. f. klin. Med., 1880, Bd. xxvi, p. 431.

**Kelling's Test.**[1]—The filtrate of the stomach contents is diluted ten times, and one or two drops of a 5 per cent. solution of ferric chloride are added. If more than one part of lactic acid to 15,000 is present, a pale, yellowish-green color will appear in the fluid. This test is not interfered with by the substances which render Uffelmann's reaction useless.

A preferable method[2] is to dilute two drops of 10 per cent. ferric chloride with 5 c.c. of distilled water, and add the gastric juice drop by drop. The presence of phosphates is less likely to interfere with the production of the proper color by the formation of an insoluble ferric phosphate than in the regular way of performing the Kelling test.

**Strauss's Test.**—A better procedure, however, is that of Strauss,[3] which permits a rough estimation of the amount of lactic acid present. A small separating funnel is employed graduated to hold 5 c.c. and 25 c.c. The funnel is filled to the mark 5 with the filtered stomach contents and then to the mark 25 with ether.

The combination is strongly shaken for a few minutes and then allowed to stand, when the ether will separate out as a clear layer above the rather milky gastric fluid. The small cock at the lower end of the funnel is then opened and the gastric contents and ether allowed to run out until the mark 5 is reached. This leaves 5 c.c. of ether in the funnel. This ether has extracted the lactic acid from the fluid. The funnel is then filled up to mark 25 with distilled water. Two drops of a 10 per cent. ferric chloride solution are added and the whole gently shaken. If 0.1 per cent. of lactic acid is present in the gastric

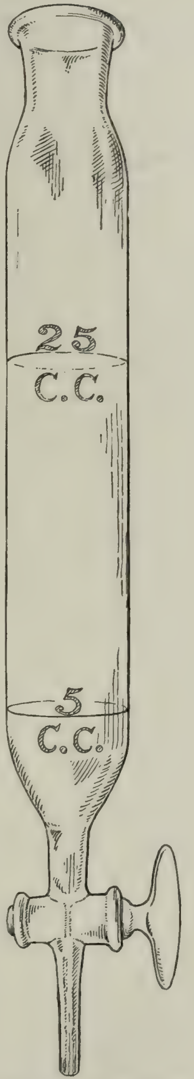

Fig. 71.—Strauss Tube for Lactic Acid Test.

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[1] Zeit. f. phys. Chemie, 1893, Bd. xviii, p. 397.

[2] *Bönniger:* Deut. med. Woch., 1902, p. 738.

[3] Berl. klin. Woch., 1895, p. 805.

contents, the water will take on a very intense yellow-green; 0.05 per cent. will still show a slight green color. Amounts smaller than this give no reaction.

### QUANTITATIVE DETERMINATION OF LACTIC ACID

The amount of organic acid present in the gastric juice when free hydrochloric acid exists in normal amounts, is very small and the quantitative determination is of no clinical importance. Lactic acid when present in considerable quantity may be approximately determined by the Strauss method or, according to Leo, by boiling 10 c.c. of gastric juice, previously acidulated with a few drops of $H_2SO_4$, until the vapors of the volatile fatty acids given off no longer redden moistened litmus paper. The fluid is then filtered, evaporated to a sirup on a water bath, filled up to the original bulk, and again evaporated. The residue is extracted four times with 50 c.c. of acid-free ether and the ether removed by evaporation. The hydrochloric acid remains in the watery residue. The residue is dissolved in water, a few drops of phenolphthalein are added, and it is then titrated with $^N/_{10}$ NaOH. Each c.c. used corresponds to 0.009 gram of lactic acid.

This method furnishes only approximately accurate results as a portion of the lactic acid is decomposed by the action of the sulphuric acid. Only a small quantity of very dilute $H_2SO_4$ should be added.

A very exact method has been devised by Boas,[1] but it is complicated, and as very accurate results are of little importance to the practitioner, the procedure need not be given here.

### QUALITATIVE TESTS FOR VOLATILE FATTY ACIDS

The volatile fatty acids in the gastric juice are chiefly butyric and acetic acids. The former can often be recognized by the strong odor of rancid butter, the latter can be determined less frequently by its vinegar-like odor.

For clinical purposes the presence of these acids is easily demonstrated by heating a few c.c. of the gastric contents in a test tube, in the upper end of which is suspended a strip of moistened blue litmus paper. The litmus paper will be reddened if any volatile acids are present.

For a more accurate identification of the volatile acids it is necessary to use from 15 to 20 c.c. of the gastric contents. Two grams of sodium sulphate are added to the gastric juice and the mixture is shaken out three times with 50 c.c. of acid-free ether. The ether is evaporated and the residue separated into two portions, one of which is tested for acetic acid, the other for butyric.

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[1] Magenkrankheiten, I. Theil, 5. Auflage, 1903, p. 212.

**1. Test for Acetic Acid.**—The residue is dissolved in water, accurately neutralized with a dilute sodium carbonate solution, and a few drops of ferric chloride are added. If acetic acid is present the fluid assumes a blood-red color and when boiled gives a brownish-red precipitate of basic ferric acetate.

Formic acid gives the same reactions, but appears very rarely in stomach contents.

**2. Test for Butyric Acid.**—The second portion of the residue is dissolved in two or three drops of water and a very small fragment of calcium chloride is added to the solution. Butyric acid is insoluble in a concentrated calcium chloride solution, so that if any of the acid is present it will be separated from the fluid in the form of small fat drops which float on the surface and have the characteristic odor of rancid butter.[1]

## DIAGNOSTIC VALUE OF TESTS FOR ORGANIC ACIDS

Lactic acid is not produced in the normal stomach during digestion, and when found in such conditions has been introduced in the food. It is evident, then, that tests for lactic acid must be made with a certain reserve unless the Boas test soup has been administered. Practically, however, the bread of the Ewald test meal does not usually contain sufficient lactic acid to give a strong reaction, so that a special meal need be given only when the determination is of special importance.

The stomach should be washed out before giving a test meal to a patient suspected of carcinoma, for in these cases there is likelihood that food from previous meals may be retained in the stomach and give the lactic acid reaction. The presence of lactic acid in the stomach contents in large amounts is suggestive but not pathognomonic of carcinoma. Its presence is an indication rather of stagnation of the stomach contents with fermentation, and also of the absence of free hydrochloric acid. The latter ordinarily checks the fermenting action of the bacilli from the food and mouth, some of which possess the power of producing lactic acid from the carbohydrates. Lactic acid is occasionally found in benign stricture of the pylorus, in dilatation of the stomach and in permanent anacidity, but usually is not present when the motility is good and free HCl is present.

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[1] *Kowarsky:* Lehrbuch d. klin. Untersuchungsmethoden, 1903, Bd. i, p. 29.

## TESTS FOR GASTRIC FERMENTS

1. **Pepsinogen and Pepsin.**—In practical work no distinction need be made between pepsin and pepsinogen, the latter being rapidly converted into pepsin in the presence of dilute hydrochloric acid, and the tests ordinarily applied indicate only the presence or absence of pepsin.

These tests are of distinctly less value from a practical point of view than those for hydrochloric acid, as in almost all cases in which a sufficiency of free HCl is present there is also an abundance of pepsin, indeed, it is generally present even if the HCl be absent, as the pepsin-producing power of the gastric mucosa is apparently less likely to be affected by disease than that of the production of free acid. For this reason the tests for pepsin are chiefly of value in cases showing no free HCl, when there may be present an advanced lesion of the gastric mucosa.

*Qualitative Test.*—Ten c.c. of the filtered gastric contents, to which has been added a sufficient amount of dilute hydrochloric acid to bring the acidity to from 0.2 to 0.5 per cent.,should that substance prove to be absent, are placed in a test tube and a small disk of coagulated egg albumin is added. The test tube and its contents are then placed in a warm place, preferably an incubator at 37° C., and left for two to three hours, with occasional shaking of the fluid. At the end of this time a disk of egg albumin 1.5 mm. thick and 10 mm. in diameter should be completely dissolved. These disks can be conveniently cut out with a cork borer from a block of white of egg which has been just coagulated by heating on a water bath. Longer boiling causes the albumin to become extremely difficult of digestion. The cylinders so obtained are cut transversely by a razor into slices 1.5 mm. thick and preserved in glycerin. Before using they should be washed off in water. Each set of disks should be carefully controlled by tests on specimens of normal gastric juice in order to determine the digestion time. This varies a good deal with each set of disks and should be noted on the bottle containing them.

Disks of dried albumin or small flocculi of fibrin colored with carmine may also be used. The digestion of the fibrin, especially, takes place very rapidly.

*Quantitative Test.*—An excellent roughly quantitative method, quite sufficient for clinical purposes, is that of Hammerschlag.[1] A 2 per cent. stock solution of egg albumin is prepared and enough hydrochloric acid added to give about 0.4 per cent. of *free* acid. Ten c.c. of the albumin solution are measured off into two separate beakers. To one are added 5 c.c. of the filtered gastric juice, to the other 5 c.c. of water. Both are placed in an incubator or left in a warm place for an hour.

The amount of albumin in each beaker is then determined by means of an Esbach albuminometer. The control, to which only water has been added, will give the percentage of albumin in the stock solution used, while in the contents of the beaker to which the gastric juice was added, only the albumin which has not been acted on will be precipitated. The difference between the two results will give the amount of albumin digested and furnish an index to the digestive activity of the gastric fluid under consideration. The method gives only approximate results.[2]

**Method of Mett.**[3]—The white of several eggs is separated from the yolk and beaten up so as to produce a uniform thin fluid, and then filtered into a flask and the latter connected with a filter pump. The fluid is kept at reduced pressure for several hours to remove any air which may be present. It is then poured into a tall beaker or a large test tube and a package of capillary tubes, 2 mm. in diameter and 10 cm. in length, are dropped into the vessel. The fluid should be of such a height that when the tubes are filled their upper ends are covered by some of the albumin solution. The beaker containing the tubes filled in this manner with the egg albumin is placed for five to fifteen minutes, depending upon the bulk of the fluid, in a water bath at 95° C., thus coagulating the albumin in the tubes. The tubes are removed and each cleaned off and the end closed with sealing-wax. To test the peptic power of digestive fluids, sections of about 2 cm. in length are cut from the tubes, using a fine file or a pair of scissors, and placed in a few c.c. of the gastric juice to be tested. At the end of ten hours the amount of albumin removed from the column by digestion is carefully determined, using a millimeter scale and a magnifying-glass. The digestive power of the juice is computed by the formula of E. Schütz,[1] according to which the relative amount of pepsin in two fluids is proportional to the square of the column of albumin digested. For example, if in two different fluids, 2 and 3 mm. respectively are digested in the same time, the amounts of pepsin present are not as two to three, but as four to nine.

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[1] Internat. klin. Rundschau, 1894, No. 39, Bd. viii, p. 1392.

[2] *Schorlemmer:* Arch. f. Verdauungskrankheiten, 1902, Bd. viii, p. 299.

[3] *Pawlow:* Die Arbeit der Verdauungsdrüsen, 1898, p. 31 (the original article by Mett is in Russian); see also *Linossier:* Journal de physiologie et de pathologie générale, tome i, 1899, p. 281; *Róth:* Zeit. f. klin. Med., 1900, Bd. xxxix, p. 1; *Schorlemmer:* Archiv f. Verdauungskrankheiten, 1902, Bd. viii, pp. 299 and 447.

It has recently been shown,[2] however, that Schütz's law does not hold with the gastric juice, unless the latter be diluted sixteen times by the addition of 15 c.c. of a twentieth normal hydrochloric acid to 1 c.c. of the filtrate of the stomach contents. The Mett tube is digested for twenty-four hours and the length of the column digested carefully measured. Normally 4 mm. is the upper limit for digestion in human gastric juice. Controls must always be made on each set of tubes made up, as the digestibility of the coagulated egg albumin varies. For clinical purposes the approximate results obtained with the undiluted juice are quite sufficient.

**Method of Thomas and Weber.**—Another means[3] of determining in a quantitative manner the action of pepsin on the proteids is based upon the digestion of a pure solution of casein in 0.2 per cent. hydrochloric acid.

A measured quantity of the gastric juice is added to a measured amount of the solution and a control with normal juice is made under similar conditions. After digestion has taken place the whole is poured into a 20 per cent. solution of sodium sulphate. The undigested casein is precipitated, collected on a weighed filter, and dried, and the weight of the filter and its contents is determined on a balance. A preliminary test of the strength of the casein solution must be made in the same manner, the difference between this amount and that obtained after digestion giving the portion acted upon by the pepsin.

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[1] Zeit. f. phys. Chemie, 1885, Bd. ix, p. 577. *J. Schütz:* ibid., Bd. xxx, p. 1, 1900.

[2] *Nirenstein* u. *Schiff:* Archiv f. Verdauungskrankheiten, 1902, Bd. viii, p. 559.

[3] *Thomas* u. *Weber:* Cent. f. Stoffwechsel- u. Verdauungskrankheiten, 1901, Bd. ii, p. 365.

It is evident that the method is suitable only for experimental work and can not be used by the practitioner as a routine procedure.

**Chymosin.**—Three to five drops of the gastric contents are added to 10 c.c. of milk, and the mixture warmed to about 35° C., either in hot water or in an incubator. If coagulation takes place in fifteen minutes chymosin is present in moderate amounts.

The zymogen appears only in gastric contents which contain no free HCl. The zymogen may be rendered active by the addition of 2 to 3 c.c. of a 1 per cent. calcium chloride solution to 10 c.c. of the faintly alkaline gastric contents, and then adding 10 c.c. of milk and warming the whole to 37° C. If coagulation takes place in a few minutes the presence of the zymogen is proven.

*The Quantitative Test for Chymosin.*—The gastric contents are carefully neutralized with decinormal soda solution and three dilutions made of the juice so diluted. One should be 1 to 10, one 1 to 20, and another 1 to 40. Ten c.c. of these dilute solutions of chymosin are then added to 10 c.c. of boiled milk, which is also accurately neutral, and the three test tubes placed in the incubator. The normal amount of chymosin will coagulate milk in a dilution of the gastric contents of 1 to 40, the zymogen 1 to 100 or 150.

The milk for these tests is conveniently kept in test tubes with cotton plugs and sterilized as if for bacterial cultures. The whole quantity of milk to be tubed should be boiled and allowed to stand overnight in a cool place until the cream has risen. The milk is then siphoned off without disturbing the layer of cream and neutralized. It is then tubed in accurately measured amounts of 10 c.c. and sterilized three times on three successive days. If the tubes are kept for a long time, the amount of milk present will diminish from evaporation and must be made up by the addition of water at the time the tube is used in the test.

**Lipase.**—Inasmuch as the secretion of a fat-splitting ferment by the human stomach has not been proved, notwithstanding the fact that it is frequently possible to demonstrate lipolysis by the gastric juice, qualitative and quantitative determinations of this ferment are of but little practical value. Its absence has been noted in cases of achylia, even when traces of pepsin are still present. It is undoubted that in many cases the presence of lipase in the stomach is due to regurgitation of the pancreatic juice through the pylorus.

For quantitative methods consult Volhard[1] and Stade[2].

### DESMOID REACTION

In order to test the digestive power of the stomach without passing the stomach tube, Sahli[3] has devised what is known as the desmoid reaction. A capsule containing 0.05 gram of methylene blue is prepared from rubber dam, a piece of which is selected measuring about 4 cm. square and 0.2 mm. thick. The capsule is tied with raw catgut, No. 00, rendered pliable by soaking in cold water. The excess of rubber beyond the ligature is cut off, taking care that the edges do not adhere. Care should also be taken to see that the capsule sinks in water and is tight. The patient swallows the capsule with or just after a full meal. The urine is collected at short intervals and examined for methylene blue (p. 586). If the greenish blue color appears in the urine within from six to twenty hours, it is taken to mean that the gastric juice contains sufficient acid and ferment to digest catgut, that substance not being digested by pancreatic juice according to Sahli.

The time of the appearance of the reaction is shortened in cases of hyperacidity, and lengthened in cases of subacidity or anacidity, as the stenosis prevents the passage of the dye into the intestine where it can be absorbed, the gastric mucosa not having such power. It is often much delayed in carcinoma of the stomach. The method does not differentiate between the various functional disorders, and does not separate cases of slowed and increased motility. In other words, it does not completely replace the use of the stomach tube with chemical analysis of the gastric contents; but a positive test shows that the digestive power of the gastric juice is approximately normal, and a negative, that hydrochloric acid is diminished or absent.

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[1] Zeit. f. klin. Med., Bd. xlii, 1901, p. 414.

[2] Beit. z. chem. Phys. u. Path., iii, 1903, p. 291.

[3] Corr.-Bl. f. schw. Aerzte, xxv, 1905, p. 241.

### NUCLEUS TEST

A test which is the reverse of the above is the nucleus test of Schmidt.[1] Inasmuch as the gastric juice does not digest the nuclear substance of cells, while the pancreatic juice does, if nuclei of ingested particles remain, after passage through the intestine, it may be assumed that the pancreatic juice is defective or absent.

Small cubes cut from the round of beef, about 0.5 cm. in diameter, are hardened in 90 per cent. alcohol for a few days, then tied up in a bag of coarse gauze and replaced in the alcohol. Before administering, the alcohol should be washed out by immersion in a stream of running water. The cubes are given, if possible, with breakfast, and the stool passed the following morning is softened with water and run through a sieve. If necessary, a moderate laxative should be given, as the intestinal bacteria are capable of affecting nuclei by the proteolytic ferment which they give off. A frozen section is made of the recovered meat ball, and is stained with hæmatoxylin-eosin in the usual manner.

Only a moderate number of cases in which this test has been employed are on record, and in general the reports are favorable. Steele,[2] however, thinks that the nuclei may be well preserved in cases in which there is a general lowering of the digestive power of the intestine, not necessarily connected with the absence of pancreatic juice.

### TESTING STARCH DIGESTION IN THE STOMACH

Starch is altered by the ptyalin of the saliva into amidulin, this into erythrodextrin, which by the same process becomes achroödextrin, the final product being chiefly maltose. Amidulin is colored blue by iodine, erythrodextrin a violet or mahogany-brown, while achroödextrin is not colored.

If Gram's solution is gradually added to a mixture of the above compounds, the achroödextrin will combine with the iodine added until an excess is present, when the brown color of the erythrodextrin will appear and finally the blue of the amidulin.

The action of the ptyalin is inhibited by the free acid of the gastric juice in a short time after the arrival of the food in the stomach, but Müller [3] has shown that from 50 to 80 per cent.

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[1] Verh. d. Kong. f. innere Med., Bd. xxi, 1904, p. 335.

[2] Univ. Penn. Med. Bull., vol. xix, 1906, p. 235.

[3] Verh. d. XIX. Kong. f. inner. Med., 1901, p. 321.

of the starch contained in the food may be in soluble form in the stomach even though the normal secretion of hydrochloric acid has taken place. In general it may be assumed that with a normal salivary ferment action a considerable quantity of the starch of a test meal will reach the stage of erythrodextrin before the ferment is checked by the free hydrochloric acid, while if free hydrochloric acid is not present, achroödextrin will be formed in large amounts. If, therefore, we add some Gram's solution or tincture of iodine to the filtrate from a test meal, and a strong blue color is immediately obtained, we may assume that hyperacidity exists and that the action of the ptyalin has been rapidly checked. If a small amount of achroödextrin is present the gastric digestion may be considered as normal; while if a large amount of achroodextrin is present and only a faint reaction for erythrodextrin is obtained, an absence or at least a marked diminution, in the amount of hydrochloric acid may be assumed. It will be noted that no consideration is paid to the possibility that variations may occur in the activity of the ptyalin; but this may be neglected, for the secretion of the salivary ferment is rarely altered by disease.

### DIAGNOSTIC VALUE OF TESTS FOR ENZYMES

As a rule, the production of the enzymes by the cells of the stomach mucosa varies far less under the influence of the nervous system than the production of hydrochloric acid. Thus the hydrochloric acid may be absent in nervous dyspepsia, in menstrual anomalies, from chronic congestion of the stomach in heart cases, etc., while during this time the cells producing pepsinogen, lipase, and the milk-curdling zymogen may be excreting a fairly normal amount of these ferments. Hydrochloric acid may be absent quite constantly in early cases of chronic gastritis, while normal amounts of the ferments are still present. Such cases are capable of much improvement after a careful course of treatment, while in those cases in which the ferments are absent or very greatly diminished a very considerable alteration in the mucosa may be assumed and treatment may be expected to be of little value. The detection of the ferments then permits the easy distinction of functional disturbances of the stomach from advanced lesions of the mucous membrane of the organ. They are, however, usually present even in advanced gastric carcinoma.

The tests for the milk coagulating ferment are especially convenient in judging of the condition of the secreting cells, as they require only a small amount of gastric juice to carry them out and no free acid, while the results are obtained promptly. Glaessner [1] has recently found that the chymosin is secreted chiefly by the fundus glands, while pepsin is derived from both the fundus and the pyloric group. He has suggested the possibility of a topical diagnosis of carcinoma based upon the diminution in chymosin which would be expected if the fundus were largely involved.

## TESTING THE MOTILITY OF THE STOMACH

An exact method of determining the motility of the stomach is still lacking. For clinical purposes the results obtained after the administration of a Riegel test meal and washing out the stomach at the end of seven or eight hours, are about as valuable as more complicated tests. A similar test can be made after the Ewald test meal by washing out the stomach two hours after the meal has been given and noticing how much food still remains. In a stomach with normal motility practically all evidence of the test meal will have disappeared. This does not prove, however, that the food has been digested; it may have passed the pylorus in a very slightly altered form.

The salol test of Ewald is based upon the supposition that salol is only split up into its components, phenol and salicylic acid, by the intestinal juices, and that no absorption of this drug takes place in the stomach. If, then, one gram of salol is given in a capsule after a meal, the bladder having been previously emptied, and the urine is tested by ferric chloride for salicylic acid, the presence of the latter should be made out in from one to one and a half hours. In cases of retarded motility and in some normal persons, two hours or more may elapse before the violet color due to the presence of the salicylic acid makes its appearance.

Instead of adding the iron salt to the urine, Einhorn suggests moistening a strip of filter paper with the latter fluid and then placing a drop of ferric chloride on the moistened slip. The edges of the drop assume a violet color and the papers may be labeled and preserved for comparison later with similar tests.

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[1] Berl. klin. Woch., 1902, p. 675.

It has been found, however, that occasionally salol is split up by the mucus contained in the stomach, so that this test has lost some of its supposed value.

The use of iodipin in this test has been suggested.[1] This substance is not decomposed in the stomach but in the upper intestine by the action of the pancreatic juice. The drug is given in a capsule and the saliva is tested every fifteen minutes for iodine with starch paper and fuming nitric acid. The reaction usually appears within fifteen to forty-five minutes in normal persons.

## TESTING THE ABSORPTIVE POWER OF THE STOMACH

The absorptive power of the stomach may be tested by giving a few grains of potassium iodide in a gelatin capsule, which has been carefully washed off after filling, and testing the saliva at intervals of two or three minutes with a little starch paste or starch paper, and some nitric acid. Instead of nitric acid, the starch paste or paper may be treated (in the dark) with a 5 per cent. ammonium persulphate solution and dried. Any iodide gives a deep blue color on this paper. In from six to ten minutes in healthy persons, a distinct violet color can be observed, and at the end of fifteen minutes a deep blue from the presence of iodine in the saliva; but the results of this test are not of great value, inasmuch as rapid absorption has been found in dilatation of the stomach and in chronic gastritis.

## DIAGNOSIS OF DISEASES OF THE STOMACH

### ULCER OF THE STOMACH

The diagnosis of ulcer of the stomach depends chiefly upon the clinical symptoms of the disease and not upon the examination of the stomach contents. The presence of blood in the washings and the high figures obtained for free acid may be suggestive; but, as a rule, it is wiser not to pass a tube on a case of suspected ulcer. The fæces often give reactions for blood.

### DILATATION OF THE STOMACH

This condition (also termed mechanical insufficiency of the stomach) gives rise to significant changes in the stomach contents. The most important of these is the retention of food particles in the stomach for twenty-four to forty-eight hours, or even longer.

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[1] *Winkler* u. *Stein:* Cent. f. inner. Med., 1899, Bd. xx, p. 849; *Heichelheim:* Zeit. f. klin. Med., 1900, Bd. lxi, p. 321.

The patients often vomit large quantities of fluid. The acidity may be diminished, and in these cases small quantities of lactic acid are found.[1] The organic acids, however, frequently disappear after the stomach has been washed several times. Some cases, especially those with most marked dilatation, show normal or even increased acid and consequently no large amount of organic acids. The ferments are apt to be diminished when the hydrochloric acid is low. Bacteria are usually very abundant, especially in advanced cases of dilatation. The amount of urine is diminished and the chlorides in that fluid are often low. The resorption of potassium iodide is delayed. On the whole the practitioner will learn more from the physical examination of the patient than from the chemical or microscopical study of the gastric contents.

### ACHYLIA GASTRICA (*Atrophic Gastritis*)

This is a rare condition, perhaps most often seen in connection with pernicious anæmia. The examination of a test meal shows that the food is not digested. Free hydrochloric acid is absent; the total acidity is low. Lactic acid may be present in small quantities. The ferments are much diminished and frequently absent entirely. The motility of the stomach is well preserved and retention of food particles is not usual. The resorption of potassium iodide is not delayed.

### CONTINUOUS HYPERSECRETION[2] (*Gastrosuccorrhœa*)[3]

The symptoms connected with the continued secretion of large quantities of gastric juice are of sufficient importance to warrant the collection of these cases under a common title, although as yet opinions are somewhat divided as to the pathogenesis of this disease.

Possibly the most generally accepted view is that it is a functional neurosis of the stomach which results in the continued secretion of abnormal amounts of gastric juice.

The objective symptoms are:

*a*. A considerable quantity of gastric juice is present in the fasting stomach. Authorities differ somewhat as to the amount which may be considered as pathological. Strauss [1] is inclined to regard 10 c.c. as an abnormal amount, and 40 c.c. as an evidence of hypersecretion; while Boas places the limit at 100 c.c. The secretion so obtained must be free from all food remnants, and should contain no sarcinæ or yeasts, nor should it give gas when mixed with dextrose and allowed to ferment at incubator temperatures. If the fluid contains food remnants or responds to the above test it is probably retained secretion due to poor motility and not hypersecretion.

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[1] *Strauss:* Zeit. f. klin. Med., 1894, Bd. xxvi, p. 514; ibid., 1895, Bd. xxvii, p. 31.

[2] *Riegel:* Zeit. f. klin. Med., 1886, Bd. xi, p. 1; 1887, Bd. xii, p. 426.

[3] *Reichmann:* Berl. klin. Woch., 1882, p. 606; 1884, pp. 21 and 768.

*b.* There is usually an increased amount of free hydrochloric acid in the gastric juice and in the stomach contents after a test meal, often rising to 60 or 80 c.c. of decinormal alkali.

*c.* Owing to the high acidity, there is diminished starch digestion after the administration of a test meal and the iodine reaction gives a dark blue color instead of the usual pale red or purple.

*d.* There is a moderate dilatation of the stomach with slight loss of motility.

*e.* The chlorides of the urine are generally reduced in amount. This reduction may be very great if the patient vomits the excessive secretion. In one case reported by Strauss the chloride secretion fell to 0.44 gms. in twenty-four hours.

### DIAGNOSIS BETWEEN CHRONIC GASTRITIS AND NERVOUS DYSPEPSIA

The differential diagnosis between chronic gastritis and a neurosis of the stomach is in typical cases a very difficult matter, occasionally quite impossible. There is no single symptom which can not be found in both. The clinical course of the disease in a neurotic person may or may not be of value. Chronic gastritis may incite or may be accompanied by neurasthenia. The examination of the stomach contents often furnishes points of value upon which the differential diagnosis may be made, if a sufficient number of examinations are carried out to eliminate the transient differences in the chemical constitution of the gastric juice.

As characteristic of chronic gastritis we find that the stomach contents in a fasting condition always contains a large amount of mucus, accompanied by epithelial cells from the mucous membrane of the stomach and clumps of leucocytes. An increase, diminution, or absence of hydrochloric acid, by itself, is equally characteristic of a neurosis or of gastritis; but the amount of hydrochloric acid secreted in a stomach the site of a gastritis, is quite constant over a period of several examinations, while neurotic cases are characterized by a very great fluctuation in the amount of acid obtained by analysis.

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[1] Untersuchungen über Magensaftfluss, Jena, 1903.

The determination of the amount and activity of the ferments secreted is of very considerable importance. In neurosis of the stomach the ferments are present in a normal amount, even though the hydrochloric acid be diminished, while in chronic gastritis the ferments are diminished in amount, especially in advanced cases. The diminution of the ferments, accompanied by an increase of the mucus of the stomach, is considered by Boas as very significant of chronic gastritis. Occasionally small fragments of the mucous membrane of the stomach may be obtained from the wash water, and a microscopic examination of these may show a chronic inflammation characteristic of chronic gastritis.

### DIAGNOSIS BETWEEN CHRONIC GASTRITIS AND CARCINOMA OF THE STOMACH

Both of these conditions may cause a very marked loss of weight and a severe anæmia. The subjective symptoms are the same for both diseases, especially as cases of carcinoma are usually accompanied by more or less chronic gastritis. This, however, is not always the case, for carcinoma occurs fairly often in a stomach which has never given the slightest evidence of functional inefficiency. Advanced cases of carcinoma of the stomach show a more severe emaciation than chronic gastritis. Occasionally, by palpation of the stomach after inflation, it is possible to obtain some evidence of a tumor, which furnishes a positive proof of the condition present. The dilatation of the stomach which occurs in carcinoma is less often seen in chronic gastritis; and the stomach in the latter condition, except for the presence of mucus, is usually empty, while in carcinoma it is regularly possible to find more or less food in the washings obtained from a fasting stomach. The presence of free hydrochloric acid speaks against carcinoma. In chronic gastritis the hydrochloric acid is usually reduced and the organic acids are not usually abundant, while in carcinoma the presence of abundant lactic acid can be determined in a large majority of cases. On the other hand, it is well known that in carcinoma of the stomach, more or less motility may persist for a long time, and that in such cases the analysis of the gastric contents may coincide perfectly with that found in chronic gastritis. In such a dilemma, the effect of forced feeding of the patient is valuable, but should be practised for a short time only, for if the condition should prove to be malignant, the delay might render surgical interference hopeless. If the weight can be increased by careful dietetic measures and remain increased for some time, and if the hydrochloric acid secretion reappears after treatment by lavage, the condition is probably one of gastritis and not of malignant disease.

### DIAGNOSIS OF CARCINOMA OF THE STOMACH

The changes in the chemical constitution of the gastric juice in carcinoma are dependent upon a number of factors. Among these may be enumerated the diminution in motility of the stomach, the change in the secreting power of the cells of the stomach mucosa and the reduction of the chloride content of the blood, both of which diminish the amount of free hydrochloric acid secreted, and the action of bacteria introduced with the food.

The diminution in the motility of the stomach is always to be expected when the carcinoma occupies the pylorus, and in these cases the stomach is rarely free from food. The same condition, however, may appear in carcinoma of the curvatures and the fundus, but not so regularly, and, as a rule, the organ is small.

The special points in the alteration of the chemical secretions of the stomach are the absence of free hydrochloric acid and the presence of lactic acid. The fluids exuding from the surface of the tumor help to neutralize free hydrochloric acid. In this respect, however, it is important to remember that the absence of free hydrochloric acid is not a necessary indication of carcinoma, for Boas has shown that out of one hundred cases of various conditions of the stomach in which no free hydrochloric acid was present, only thirty-one proved to be carcinoma. The free hydrochloric acid may be present in normal amounts, or even in excessive quantities, when the tumor is small and occupies the pyloric region. The excess of hydrochloric acid present in these cases is not always an indication that carcinoma has arisen on the base of an old ulcer, and one must also remember that hydrochloric acid may be absent in ulcers of the stomach and absent also in connection with carcinomata of neighboring organs, for example, of the gall-bladder, pancreas, and duodenum.

The presence of lactic acid in the stomach is a much more valuable indication than the absence of hydrochloric, for it has been shown that 85 per cent. of patients showing a marked amount of lactic acid on chemical analysis have malignant changes in the walls of the stomach. Special series of cases have shown that 93 per cent. of the patients in whom a positive reaction for lactic acid could be obtained had carcinoma of the stomach. To offset this, lactic acid in considerable quantity has been found occasionally in achylia gastrica, and in advanced chronic gastritis with atrophy and dilatation of the stomach, especially when accompanied by a benign stenosis of the pylorus. In general, however, simple stenosis of the pylorus is accompanied by dilatation of the stomach and an increased secretion of hydrochloric acid, hence no lactic acid.

Ewald has called attention to the frequent occurrence of lactic acid in patients suffering from atrophy of the mucous membrane of the stomach in connection with pernicious anæmia. In those cases in which considerable quantities of hydrochloric acid are present we naturally expect no lactic acid.

Another diagnostic point which is of great value is the discovery of small tumor particles in the washings from the stomach. This, unfortunately, is a very late symptom and indicates that the growth has in all probability extended beyond the point of successful operative interference. The presence of small quantities of blood and pus in the stomach contents is of corroborative value. Blood can be demonstrated in the washings in nearly one-half the cases, and in the stools in nearly all persons the subjects of gastric carcinoma. This shows that the tumor ulcerates early and small hæmorrhages take place from the surface. In all probability, so long as the motor activity of the stomach remains good, these blood traces are rapidly passed on through the pylorus and hence are not always demonstrable in the stomach washings.

Microscopic examination of the stomach contents in carcinoma of the stomach may furnish an additional point—that is, the presence of the Boas-Oppler bacilli. which are connected with the formation of lactic acid.

# PART III

# THE FÆCES

## I. GENERAL CONSIDERATIONS

THE fæces are composed of a mixture of undigested food residues, of such portions of the gastric, pancreatic, hepatic, and intestinal secretions as remain unabsorbed, and of desquamated epithelium, cell débris, and bacteria. The gastric secretion has already been considered. Its acid reaction incites the secretion of the pancreatic juice.[1] The pancreatic tryptic ferment is excreted in the form of a zymogen which is activated by the enterokinase[2] of the intestinal mucosa, while the fat-splitting and starch-digesting ferments are active when secreted.

The presence of food in the duodenum causes an outflow of bile. The bilirubin of that fluid is altered by the intestinal bacteria into hydrobilirubin, a compound closely related to, if not identical with urobilin. This substance gives the brown color to the fæces.

The cholesterin derived from the bile can be demonstrated in the fæces of children or adults on a milk diet. If, however, the bacterial fermentation is active, the cholesterin is reduced to a closely related compound, coprosterin, just as bilirubin is reduced to hydrobilirubin.

It is probable that the intestinal mucosa secretes a ferment, erepsin,[3] which is capable of splitting proteoses into the amino bodies, such as leucin, tyrosin, lysin, etc. A ferment which splits lactose is present in the intestinal tract of infants, this form of sugar not being acted on by the pancreatic ferments.

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[1] *Glaessner* (Münch. med. Woch., 1903, p. 491) has been able to observe an increase in the pancreatic secretion after the taking of hydrochloric acid in a patient with a pancreatic fistula. *Bayliss* and *Starling* (Jour. of Phys., 1902, vol. xxviii, p. 325) think that the stimulus to the pancreatic secretion is not directly due to the acid, but to a ferment, "secretin," which is set free by the action of the acid on the intestinal mucosa, is absorbed into the circulation, and then stimulates the pancreas.

[2] A term applied by *Pawlow* to the ferment of the intestinal mucosa, which has the power to render active the proteolytic ferment of the pancreas.

[3] *Cohnheim:* Zeit. f. phys. Chemie., 1902, Bd. xxxv, p. 418.

The presence of large numbers of bacteria in the lumen of the intestine is a normal condition, though it has been shown that sterile animals can live.[1] It is probable, however, that the bacteria assist in the process of digestion by giving off ferments which act upon the food and are necessary for the complete and normal development of the organism.[2] Excessive putrefactive action, however, promptly gives rise to functional disturbances of digestion, best evidenced by a great increase in the urinary excretion of two products of bacterial activity on proteids, indol and skatol.

*Meconium* is the name applied to the dark, pasty substance passed during or after delivery. It consists chiefly of thickened bile and substances swallowed by the infant. Chemically it contains glycocholic, taurocholic, and fellic acids, cholesterin, lecithin, bilirubin and mucus. Microscopically, hair and cells from the epidermis and intestinal mucosa are not infrequent; fat globules and cholesterin crystals are abundant. Bilirubin crystals may be found.

## II. MACROSCOPICAL EXAMINATION

The macroscopic examination of the fæces concerns chiefly the consistence, color, odor, and the amount of the fæces excreted, together with the recognition of the presence of mucus, pus, blood, or parasites. It also includes the search for and the identification of gall-stones which may have entered the intestine from the bile passages.

The technique of the examination is simple. As a rule, no especial preparation of the material is required except in the search for gall-stones, when the fæces should be softened with a large amount of water and washed through a fine sieve. Small amounts of mucus and of connective-tissue fragments are most easily recognized by rubbing a little of the stool in a glass mortar with a little water or spreading out the stool between two glass plates.

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[1] *Thierfelder* and *Nuttall:* Zeit. f. phys. Chemie, 1895, Bd. xxi, p. 109. Ibid., 1896, Bd. xxii, p. 62.

[2] *Schottelius:* Arch. f. Hygiene, 1902, Bd. xlii, p. 48.

It is convenient, however, when examining the fæces to employ some form of washing apparatus to separate the coarse particles from the fine granular débris and bacteria.

Boas[1] has devised a small stool sieve consisting of two hemispherical compartments which can be fastened together by a bayonet catch. A fine sieve in the lower compartment retains the food fragments, gall-stones, etc., while the finer particles pass through and are washed out of the apparatus. In this way a specimen for study may be obtained in about twenty minutes.

Strauss[2] suggests the use of a glass percolator or irrigation jar which is supported on an iron tripod and is connected with the water supply by a rubber tube attached to the aperture at the lower portion. The stool to be washed is placed in the jar, a gentle stream of water is turned on, and the top of the vessel covered with a weighted sieve, the meshes of which are about 1 mm. in diameter.

Einhorn[3] advises the use of an ordinary flour sifter, which can be obtained at any hardware shop for a few cents. The stool is placed on the sieve, the latter immersed in a vessel of water or held under the tap, and the handle is turned slowly.

The **amount** of the fæces excreted depends chiefly upon four factors:

*a. Quantity and composition of the food.* It is evident that the amount of fæces will vary with the amount of food taken into the intestinal tract, and also that the coarser varieties of food containing much cellulose will furnish a more bulky fæcal mass than meat, which is largely digested and absorbed.

*b. Intestinal débris.* A very considerable part of the fæces is supplied from the débris from the intestinal mucosa, which may amount to one-sixth of the total quantity of the fæces passed, or 4 grams of dried residue out of a total 25 grams per day.[4]

*c. Bacteria.* A considerable proportion of the bulk of the fæces, both in health and in disease, is made up of bacteria. It has been estimated by Strasburger[5] that approximately 8 grams of bacteria weighed dry are passed per day, when the total weight of the dry fæces is 27 grams. That is, nearly one-third of the normal fæces is composed of bacteria. Intestinal disturbances favoring the growth of bacteria may increase the amount to 14 grams, while in simple constipation the bacteria in the stools diminish and may amount to only about 5 grams. In jaundice with fatty stools, the number of the bacteria is greatly diminished.

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[1] Deut. med. Woch., 1900, p. 583.

[2] Fort. d. Med., 1902, p. 937.

[3] Deut. med. Woch., 1901, p. 159.

[4] *Schmidt* u. *Strasburger:* Die Fæces des Menschen, Berlin, 1901.

[5] Zeit. f. klin. Med., 1902, Bd. xlvi, p. 413.

*d. The condition of the digestive organs.* The amount is also influenced by the condition of the digestive organs. For instance, if the normal amount of fat appearing in the stools be assumed to be 7 to 8 grams, this amount may be increased 50 per cent. by obstruction of the common bile duct.

The bulk of the fæces actually passed may be increased though the solid residue remains the same, when the peristaltic action of the bowel is much increased, thus transferring the fæces to the rectum before the usual amount of water is absorbed by the mucous membrane of the colon. If the peristalsis is diminished, the fæcal matter remains longer than normal in the bowel and may form an inspissated mass containing but little water.

The amount may also be increased by admixture of pathological substances, such as mucus secreted by the intestinal mucosa. Normally the amount of the fæces may be considered as from 100 to 150 grams, corresponding to 15 to 25 grams of dried substance.

The **frequency** depends much more upon the peristaltic activity of the small intestine and the irritability of the rectal mucosa, than upon the amount of the fæces passed, and so becomes an evidence of either inflammation of the intestine or a neurosis.

The **consistence** of the fæces determines the form, which is usually cylindrical. Tape-like stools are considered as an evidence of a stricture of the rectum; but such fæcal masses may be seen when the intestine is normal, or the stools may be normal in form when a stricture is present. The normal stool in adults is always formed, while that of infants under the normal conditions of milk feeding is quite soft and pasty.

The **color** of the stools is yellow or brown with a mixed diet. Variations are dependent upon the nature of the food. For example, the stools passed by patients on a milk diet are pale, while those passed by persons consuming large quantities of meat are very dark, often nearly black, due to the presence of hæmatin. A pale yellow or whitish stool may be due to an excess of fat.

The color is also dependent upon the digestive fluids, notably the bile, clay-colored stools being often seen in biliary obstruction. The normal color of the fæces is due to the presence of hydrobilirubin, a reduction product of the bilirubin of the bile. This substance, which is considered as the same as the urobilin found in the urine, is sometimes reduced still further to a colorless substance, leucohydrobilirubin, leaving the stools almost colorless. If such colorless stools be exposed to the air they will turn brown, owing to the oxidation of the leucohydrobilirubin back to the colored compound.

Occasionally the stools may also be colored by bilirubin and give Gmelin's reaction. This is a normal condition in the stools of infants, in whose intestine the process of reduction has not yet assumed the proportions that it does in later life. The color is to be distinguished from that due to hydrobilirubin, by the bright yellow tint caused by the bile pigment. The green color seen in the stools of infants is produced by the oxidation of the bilirubin to biliverdin. The stools of children fed on artificial foods contain, as a rule, only hydrobilirubin. It has been claimed that in rare cases the green color may be due to the presence of bacteria capable of forming a green pigment.

The color of the fæces may be due to blood, drugs, or substances of a pathological nature present in the bowel. The stools are of a blackish color after eating considerable quantities of blueberries. Iron and bismuth compounds color the stools a gray or black, probably due to the formation of oxides of these metals, and not, as is generally assumed, to the formation of the corresponding sulphides. In the case of iron, the darkening takes place after the stool is passed. After taking methylene blue by mouth, the fæces occasionally assume a pale bluish-green color on exposure to the air. Green stools colored with biliverdin or urobilin[1] are seen after taking calomel.

**Pus, mucus,** and **blood** may alter the color of the stools, especially the presence of blood. The red color due to hæmoglobin is found only when the hæmorrhage has taken place from a point near the lower end of the bowel. The black or brown stools seen after an intestinal hæmorrhage are colored by hæmatin produced by the action of the intestinal contents on the hæmoglobin.

Care should be taken to test for the presence of blood pigment in doubtful cases, and to exclude error by questioning the patient to see if large amounts of meat or any food containing blood have been recently ingested, as the reaction for hæmatin may easily be obtained under such conditions.

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[1] *v. Jaksch :* Klin. Diag., fifth edition, Wien, 1901.

## III. MICROSCOPICAL EXAMINATION

Fluid fæces may be examined without further treatment. Firm masses should have a small particle removed with a stiff platinum loop or spear. The fragment should be spread out on a slide in some physiological salt solution or formalin, and covered with a large cover glass. If a thorough search for large fragments of food is to be made, the finer particles should be washed away in one of the sieves mentioned in the second section of this chapter. As the eggs of the intestinal parasites are small enough to escape through the sieve, it is necessary when searching for them to examine the untreated stool. Very thin preparations should always be made. It is well to use a large slide, say two by three inches, and a cover glass of corresponding size, and spread the fæces out in a very thin layer, the amount of fluid added depending upon the consistency of the stool. The examination for an especial morphological element in the stools is often facilitated by making a very thin watery emulsion of the fæces and then allowing the mixture to settle. Coarse vegetable remnants, stone cells, and crystals form the lower layer, parasite eggs are in the middle layer, while the upper is composed of the bacteria, fat needles, light vegetable cells, etc.

If the odor is extremely offensive, some 4 per cent. formalin may be added.

The examination includes the search for:

1. Food remnants.
2. Elements from the intestinal wall.
3. Crystals.
4. Bacteria, parasites, and eggs.

### FOOD REMNANTS

Muscle fibers are found in all normal stools, even for a number of days after the taking of meat has ceased. The fibers rarely show any striation, or at least well-marked striation, as they do in the stomach, but consist of yellow oval masses which are highly refractile and transparent. The constant finding of large numbers in afebrile patients, especially if mucus is also found, indicates a catarrhal enteritis or pancreatic disease.

Elastic and connective tissues are often found in stools. They are derived from meat and are not always easy of identification. The elastic fibers are usually highly refractile and have a tendency to curl up. Connective tissue forms irregular strands occasionally showing the morphology of the tissues.

Starch cells are not usually found free in the normal fæces in a form recognizable by the microscope, unless some Gram's solution is allowed to run in under the cover, when there may often be found irregular, bluish masses, evidently made up of partly digested starch grains. If the starch of the food is entirely

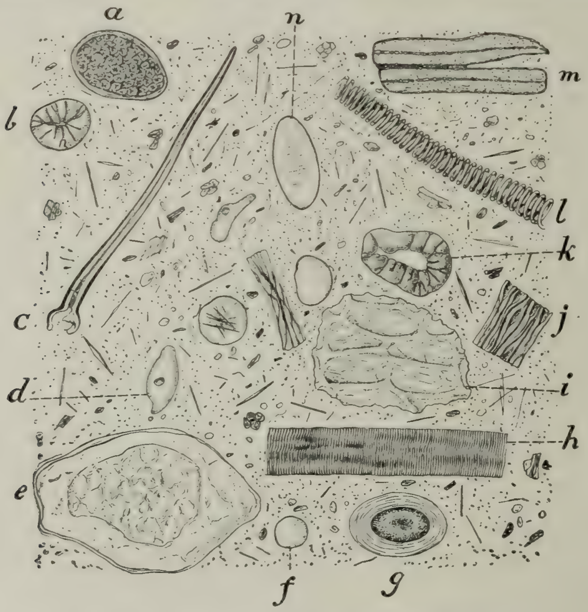

FIG. 72.—MORPHOLOGY OF NORMAL FÆCES.

*a*, Vegetable cell containing chlorophyl; such cells are often mistaken for the eggs of intestinal parasites; *b* and *k*, stone cells; *c*, vegetable hair; *d*, large starch granule; *e*, potato cell; *f*, fat globule; *g*, bile-stained calcium salts; *h*, muscle fiber; *i*, flat surface cells from vegetables; *j*, palisade cells from vegetables; *l*, spiral water-vascular tube from rhubarb; *m*, flat vegetable cells; *n*, partially digested meat fiber. Scattered through the specimen are numerous needles of fatty acids and soaps.

uncooked, then a considerable amount will appear in recognizable form in the stools. Starch is never bile stained. Unaltered starch is often seen in masses of cells from potato or other starchy vegetables. Well-formed starch grains are occasionally found in the fæces and are derived from the starch contained in dusting powders often used to prevent irritation about the anus.

Fat appears in the form of fat globules which can be blackened by osmic acid or stained red by an alcoholic solution of Sudan III or scharlach R. Fat globules are sometimes seen covered with needle-like crystals of the insoluble soaps (Fig. 72). Fatty acid needles are also seen in the form of delicate short rods resembling bacteria. These crystals melt into globules when the slide is gently heated, which differentiates them from the lime soaps. The soaps of calcium and magnesium, chiefly the former, also appear in the form of needles, but these are coarser than the fatty acid and are frequently arranged in bundles and sheaves. When fatty acid or soap needles are treated with the fat stains mentioned above they take the color, though not so deeply as the neutral fat, and then develop globular swellings, and may finally soften into a spherical mass.

Coagulated albumin and casein masses may also be found in the stools, especially of those who are on an exclusively milk diet or whose food contains a large amount of egg albumin. These fragments are usually bile stained and of small size. The casein is soluble in 5 per cent. HCl; the albumin gives the xanthoproteic reaction with $HNO_3$. Coagula composed of casein or mucus plus fat, fatty acids and insoluble soaps may be found in the dejecta of infants suffering from gastro-intestinal indigestion. The large fragments in the stools of infants which are usually assumed to be casein may be masses of fat needles and bacteria.

Débris from food remnants is always present in large amounts in the fæces, and the source of individual fragments is often impossible to determine. They are often bile stained. Some of the microchemical reactions given below may be of value in the identification.

### VEGETABLE CELLS

The stools of persons on a mixed diet contain a great variety of vegetable cells, which can be best identified by the fact that the cell wall has a double contour, thus distinguishing them at once from animal cells. Many of these cells contain chlorophyl or numerous starch granules. The cells from plants can also be recognized by the reaction for cellulose, which consists in a purple coloration produced by a solution of zinc chloride when it is allowed to act on cellulose which has previously been treated with some Gram's solution.

This reaction can be obtained from all the softer cells of the plant tissues, and from the layers of flat cells which cover the surface of plant leaves when not too old.

It can not be obtained from such cells as have undergone a change to lignin, as, for instance, in the spiral vascular tubes from rhubarb (Fig. 72, *l*) and the so-called stone cells from pears, dates, or pepper (Fig. 72, *b* and *k*). Lignin turns a violet red when treated with an alcoholic solution of phloroglucin and some HCl. It also takes a bright red when treated with safranin.

Many of the superficial leaf cells do not give either of the above reactions, but have been altered so that they turn yellow when treated with strong NaOH. The change is the same as that which takes place in cork cells.

A more detailed description of those cells is not necessary here. The student must identify them from normal stools, with the aid, if necessary, of the reactions given, until he is not likely to mistake them for parasites. Especially important in this connection is the identification of pollen, vegetable cells (Fig. 72, *a*) and certain mold spores which are found in wheat flour and resemble the eggs of the tapeworms.[1] The presence of a certain type of vegetable remnant in the fæces is no evidence that it has been recently ingested. Fragments of this sort may not be passed for days after they have been swallowed.

## ELEMENTS FROM THE INTESTINAL WALL

**Leucocytes** are found in small numbers in the normal stool and in large quantities in the stools of persons suffering from catarrhal conditions of the bowel. Large masses of pus may be found when an appendical or pelvic abscess discharges into the bowel or in severe ulcerative lesions of the intestine.

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[1] For full details on the subject of the morphology of the stools the student should consult *van Ledden-Hulsebosch:* Makro- und mikroskopische Diagnostik der Menschlichen Exkremente, Berlin, 1899.

**Red blood cells** are never found unless the hæmorrhage is from a point very near the anus, as they are quickly destroyed by the intestinal secretions.

**Epithelium** of two types is met with. Flat cells may be found, derived from the anus or from the cells of a new growth, but this find is very rare. Cylindrical epithelium is frequently present. Beaker cells filled with mucus are rare. All types of epithelium may show fatty degeneration.

**Mucus** is microscopically characterized by its clear, slightly striated structure. It often contains many included leucocytes and epithelial cells. Great care should be taken not to confuse with mucus the large, starch-containing cells derived from potato.

### BACTERIA, PARASITES, AND EGGS

**Molds, yeasts,** and **sarcinæ** are occasionally found in the fæces. The sarcinæ are especially abundant in the fæces of persons suffering from dilatation of the stomach with a growth of sarcina ventriculi in the dilated organ. The sarcinæ give the cellulose reaction with zinc chloride and iodine.

**Bacteria**—The bacterial flora of the intestine is exceedingly large, and the identification of the various saprophytes and pathogenic bacteria is a matter of great difficulty. The study of stained smear preparations is of but little practical value. The Bacillus butyricus may occasionally be identified in normal and pathological stools by its size and the blue color which it assumes when treated with iodine, but the find is of no clinical interest.

The separation of the members of the typhoid, colon, and dysentery groups has become possible by the introduction of new procedures, notably by the modified culture media which we owe to Hiss; but the methods are still too complicated for the practitioner and require the skill of the trained bacteriologist. Incomplete bacterial determinations on a single medium are worse than useless.

Attention has recently been called to the demonstration of the tubercle bacillus in the fæces as a diagnostic procedure in cases of intestinal tuberculosis. The method is only of very limited value, for tubercle bacilli can be found in the fæces of those patients with phthisis who are not careful to expectorate all of the sputum brought up into the mouth, while the difficulties connected with the morphological differentiation of the tubercle bacillus from the timothy bacillus, which is present in the intestinal contents, will always limit the value of the results. Smegma bacilli may also be found inside the anal opening, so that Pappenheim's method should always be used in staining smears for tubercle bacilli. Unless acid resistant bacilli are found constantly and in large numbers in the pus and débris derived from the ulcers of the intestine, the results can not be considered of any diagnostic value. If such masses can be found, it is best to inject some of the material into the groin of a guinea-pig.

Parasites and eggs will be considered in a later section.

## CRYSTALS

1. **Hæmatoidin** crystals have been seen after hæmorrhage into the bowel either from rupture of a vessel or during the course of an acute entero-colitis. They are yellowish needles, arranged singly and in bundles, and are soluble in chloroform.

2. **Cholesterin** crystals are rarely present. They can be recognized by their rhombic form and reaction with sulphuric acid and Gram's solution.

3. **Charcot-Leyden** crystals are to be found with some frequency. They assume the form of colorless, octahedral crystals of considerable length, and are to be distinguished from fatty acid crystals, which they resemble slightly, by their easy solubility in HCl. They are especially abundant in the stools of patients harboring the various intestinal worms.

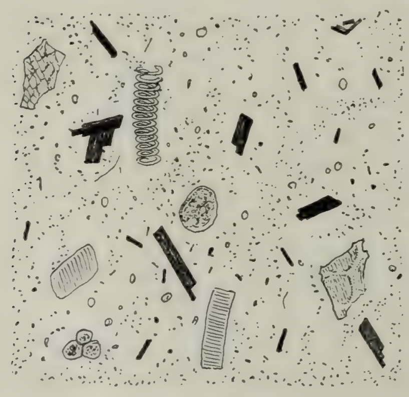

Fig. 73.—Bismuth Crystals in Stool.

4. **Fatty acid** crystals and the needles of the insoluble soaps are frequently found. The former are soluble in alcohol and melt when the slide is gently warmed; the latter do not respond to these tests.

5. **Calcium phosphate crystals** are occasionally seen. They are thick, irregular crystals united by one extremity into a rosette

form. Calcium salts of unknown composition may also be found as yellow, oval, or spherical bodies with concentric structure and often bile-stained (Fig. 72, *g*).

6. **Triple phosphate** crystals are abundant in alkaline stools and are identical with those seen in the urine.

7. **Calcium oxalate** crystals resemble those seen in the urine.

8. **Bismuth** crystals can be found abundantly in the stools after a dose of bismuth subnitrate. They are of an irregular rhombic form, with notched edges, and are very dark in color or even black. They are not the sulphide, but the suboxide of bismuth.

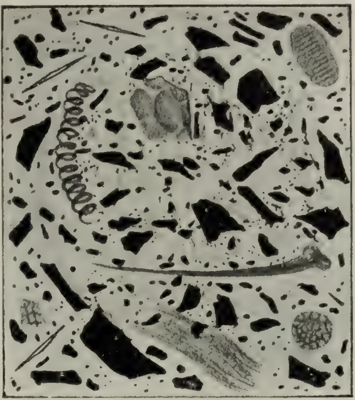

FIG. 74.—CHARCOAL FRAGMENTS IN STOOL.

9. Charcoal when taken by mouth appears in the stools in the form of irregular black masses, usually coarser than bismuth crystals.

## IV. TESTING THE DIGESTIVE POWER OF THE INTESTINE

An attempt has been made to determine the digestive capacity of the intestinal tract by the administration of a test diet, on the same principle as the administration of test meals to determine gastric activity. Schmidt and Strasburger,[1] who have largely contributed to our knowledge of the subject, use the following series of meals to test the digestive power of the intestine :

In the morning, 500 c.c. of milk and 50 grams of zwieback. If milk is not well borne, 500 c.c. of cocoa can be used, composed of 20 grams of cocoa powder, 10 grams of sugar, 400 c.c. of water, and 100 c.c. of milk.

In the middle of the morning, 500 c.c. of oatmeal gruel, made up of 40 grams of oatmeal, 10 grams of butter, 200 c.c. of milk, 300 c.c. of water, and 1 egg. The mixture should be strained.

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[1] Die Faeces des Menschen. Berlin, 1901. Also *Schmidt:* Die Funktionsprüfung des Darmes. Wiesbaden, 1904.

Dinner is composed of 125 grams of chopped beef, which has been superficially broiled, using 20 grams of butter for this purpose. Also 250 grams of mashed potatoes, made up of 190 grams of potato, 100 c.c. of milk, and 10 grams of butter.

In the afternoon, the same as the morning meal.

In the evening, the same meal as that given in the middle of the morning.

This diet contains about 102 grams of albumin, 111 grams of fat, and 190 grams of carbohydrate. The patient is to be kept on the diet for about three days or even longer, until a stool is obtained which is certainly derived from it. Under normal conditions this occurs at the second defæcation after the beginning of the test. To mark this point, the patient should take a capsule containing 3 cg. of powdered carmine. This substance passes through the intestinal tract unaltered and marks off the corresponding point in the fæces.

The specimen of fæces obtained from this diet should be examined as follows:

The color, consistence, and odor should be noted. The stool should then be softened with dilute formalin or water and spread out in a thin layer in a hard rubber photographic tray and the various macroscopical remnants studied in detail. Mucus, remnants of connective tissue and tendon derived from the beef, fragments of muscle tissue, remnants of potato, and crystals of ammonium magnesium phosphate can be determined by the macroscopical examination.

Microscopical preparations should then be made, the first without the addition of anything but dilute formalin or water. The second should have a small drop of acetic acid added to it, and is then heated over a flame until it boils, and covered with a cover-glass. To the third should be added some tincture of iodine or a strong solution of iodine in potassium iodide.[1]

In normal fæces, the first specimen will contain (*a*) some muscle fragments of oval form of a pale yellow color, usually without striations; (*b*) irregular yellow masses of calcium soaps or occasionally colorless masses of the same; (*c*) a moderate number of potato cells either empty or containing a few remnants of starch granules; (*d*) a few fragments of husks from the oatmeal gruel.

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[1] Iodine, 1; potassium iodide, 2; distilled water, 50.

If cocoa has been taken instead of milk, brownish remnants of the cocoa powder may be found in the stools. In the preparation which has been heated and allowed to cool, numerous fatty acid masses may be seen scattered throughout the entire preparation and the nodules of lime soaps will have disappeared. If the preparation is again heated and examined under a microscope while still hot, the fatty acid nodules melt together into drops, which again crystallize as the preparation cools.

In the third preparation to which iodine has been added, a few starch granules contained in the potato cells will be stained blue and occasionally some molds may be seen which have assumed a blue color.

If the intestinal digestion is imperfect or if pathological conditions exist, the first specimen will show (*a*) large fragments of muscle tissue with well-marked transverse striations and clean-cut transverse ends to the cylinders; (*b*) numerous drops of neutral fat; (*c*) very abundant fatty acid and soap nodules; (*d*) large numbers of potato cells; (*e*) eggs of parasites, mucus, pus, blood, etc.

In the acetic acid preparation under pathological conditions the amount of fatty acid may be very large.

In the slide treated with iodine will be found large numbers of undigested starch granules, numerous blue or yellow molds, and rod-shaped bacilli and yeast cells.

The test stool should also be examined chemically. The reaction should be determined by rubbing up a little of the stool with distilled water and dipping into the mixture a strip of red and a strip of blue litmus paper. Normally the reaction is amphoteric, but may be faintly acid or faintly alkaline.

Other portions of the stool should be subjected to the bichloride test by being rubbed up with a saturated solution of mercuric chloride in a small glass dish. The dish is then covered and allowed to stand until the next day. Normal stools are colored a brilliant red, which is brighter if the stool is fresh and less intense if the specimen is old. Greenish particles are pathological. They are an evidence of the presence of unchanged bile pigment.

The fermentation test should also be carried out in the apparatus shown in Fig. 75. A portion of untreated stool about the size of a walnut is placed in the vessel *a* and stirred up with a little water, and the rubber stopper inserted, being careful not to inclose any air bubbles as it is placed in position. Tube *b* is then filled with water; *c* is left empty. The apparatus is then placed for twenty-four hours in an incubator at 37° C. Any gas which develops collects in *b* and a corresponding amount of water is driven over into tube *c*, the height of the fluid being noted. The vessel *a* is then opened and the reaction of the contents determined with litmus paper. In normal stools only a small amount of gas is formed under these conditions and the original reaction is only slightly changed. If sufficient gas is developed to fill tube *c* a third or more with water, the fæces contain sufficient undigested carbohydrates or albumin to warrant the conclusion that the intestinal digestion is imperfect. If the carbohydrate is in excess, the gas evolved will smell strongly of butyric acid. If albumin is present in large amount the odor is that of putrefaction.

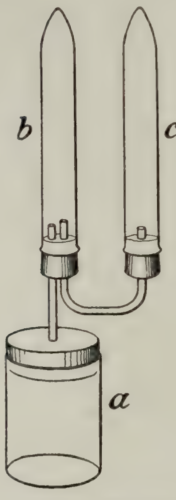

Fig. 75.—Fermentation Apparatus for Fæces.

## V. THE FÆCES IN DISEASE

**Acute entero-colitis.** The movements are usually thin and contain small particles of mucus intimately mixed with the fæcal matter. If the small intestine is chiefly involved, the mucus is often bile-stained and contains numerous cylindrical epithelial cells. When the inflammation is most active in the large intestine the mucus is usually in clear masses and not bile-stained. Small amounts of blood are not uncommon; large hæmorrhages are very rare.

**Chronic entero-colitis** may show either constipation or diarrhœa. The nature of the mucus is the same as in the acute forms, but when there is no diarrhœa it is often necessary to wash out the colon and to examine the washings for mucus. If the latter be present in large amounts, the large intestine is probably the site of the lesion.

**Membranous enteritis** is characterized by the passage of large quantities of mucus, which may be tubular, as a cast of the bowel, or may be in the form of a loose membrane. It is composed of much mucus and albumin, in which are imbedded a moderate number of degenerated epithelial cells and leucocytes, and occasionally a few Charcot-Leyden crystals. (See Fig. 76, page 321.)

**Acute gastro-enteritis** in children is usually accompanied by diarrhœa. The stools are frequent and small. They are of a pasty consistence and contain large amounts of fatty acids. In the so-called fat diarrhœa, the needles of acid form white glittering streaks on the surface of the stool. The stools are usually greenish from the mixture of bile. Blood is seen in the severe cases, and at times the stools may be nearly pure serous exudate from the bowel, mingled with leucocytes and epithelium.

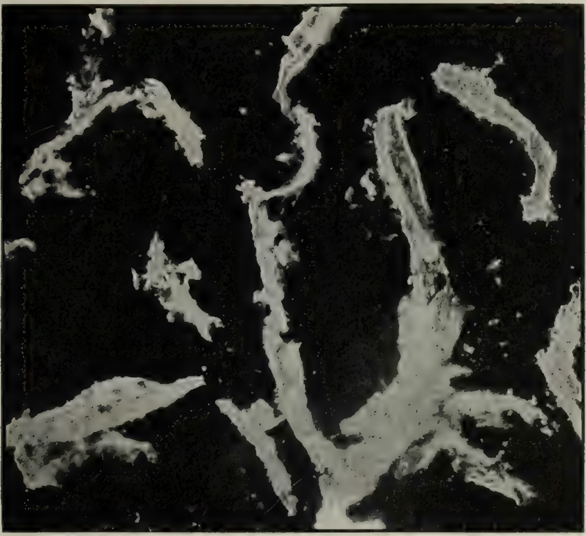

Fig. 76.—Mucus from a Case of Membranous Enteritis.
(One half natural size.)

**Ulcer of the intestine.** The three cardinal evidences of ulcer are the presence of blood, pus, and shreds of tissue in the stool. Blood is most frequent in dysenteric and typhoid ulceration, less so in tuberculous and follicular ulcers. If the pus is mixed with blood, it is much more likely to have originated from an intestinal ulcer than if it is in a pure condition; for pure pus is very probably from some abscess near the intestine, and not from a process in the lumen. This, however, is not absolute.

**Dysentery** is characterized by very small and frequent stools containing very little fæcal matter and much mucus, pus, and blood. When the ulceration of the intestine reaches an advanced grade, fragments of the necrotic mucosa and masses of fibrin may be found in the fæces. At different stages of the disease, the proportions of the mucus, pus, and blood may vary. At first a moderate amount of fæcal matter is present, often coated with mucus and a little blood. Later the blood becomes more abundant and, as the ulceration of the intestine advances, pus and necrotic fragments begin to appear. Stools composed of pus or blood alone may be seen in severe cases. In the latter, the stool is fluid and of a chocolate color from the blood pigment.

In many cases of acute dysentery, bacilli have been isolated, which are assumed to be the inciting agents in this disease.[1] The original observations were made by Shiga[2] on an epidemic of dysentery in Japan. Kruse[3] isolated the same organisms in Germany. Flexner[4] obtained a closely related organism from the Philippine Islands, which differs from the true Shiga bacillus in its agglutinative reactions and cultural characteristics. A third bacillus has been isolated by Hiss and Russell[5] from a case of acute enteritis in a child, which also differs slightly from the bacilli obtained by Flexner and Shiga.

Duval and Bassett[6] have been able to isolate bacilli of the Flexner type from the stools of infants suffering from acute enterocolitis.

At present it seems probable that the bacilli above described have some causal relationship to acute dysentery, but except for an accidental laboratory infection reported by Strong, in which a person developed dysentery after swallowing a culture, and the agglutinative power of the patient's blood serum on the bacilli isolated from the stools, we have as yet no facts which warrant the conclusion that these bacilli are the sole cause of the disease and that we may use the bacterial findings as a means of diagnosis. At present the diagnosis of dysentery should be made from the clinical picture, and not from the bacteria isolated from the stools.

In the masses of mucus of a number of the more chronic types of dysentery, amœbæ may be found, which are supposed by many to have a causal relation to the disease. Various types of amœbæ may be found in the stools of persons in perfect health and in the stools of those suffering from a simple diarrhœa, and a morphological separation of the two forms is often difficult. See page 339 for further details.

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[1] For a review of the bacteriology of the subject, see *Lentz:* Handbuch der pathogen. Mikroorganismen, 1903, Bd. ii, p. 309.

[2] Cent. f. Bakt., 1898, Bd. xxiii, p. 599. Ibid., 1899, Bd. xxiv, p. 817.

[3] Deut. med. Woch., 1900, p. 637.

[4] Cent. f. Bakt., 1901, Bd. xxx, p. 449.

[5] Medical News, 1903, vol. lxxxii, p. 289.

[6] Cent. f. Bakt., 1903, Bd. xxxiii, p. 52.

In **typhoid fever** the bowels are often somewhat constipated in the first week and the stools offer nothing of interest, but in the second and third weeks the characteristic pea-soup stools usually appear.

Small amounts of blood should be carefully looked for in the stools of the latter part of disease, by means of Weber's test, as they frequently precede a hæmorrhage by a considerable time, and the early diagnosis of the condition allows time for the proper medical or surgical treatment.

Hiss,[1] in 1897, using a special medium devised for the purpose isolated the typhoid bacillus in about half of a series of unselected cases of typhoid fever, as the result of a single examination. In a series of nineteen hospital cases in the febrile stage of the disease the typhoid bacillus was isolated in seventeen. The bacilli occur most regularly in the stools about the middle of the second week.

Higley[2] by the same method obtained the bacilli in twenty cases out of twenty-one, chiefly before the second week. Drigalsky and Conradi[3] were successful in twenty-five out of fifty cases before the appearance of the rash and the Widal. Hayaschikawa[4] obtained 60 per cent. of positives. Great technical skill is required for this method, and it is not so generally useful diagnostically as the blood culture method.

In **cholera,** comma bacilli may be found in the masses of mucus, but the finding must always be confirmed by cultures.

In **carcinoma** of the rectum the stools may contain blood and mucus. The diagnosis is made, however, by palpation of the growth or by the use of the speculum. In carcinoma of the upper intestine there is nothing characteristic in the stools except occult blood.

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[1] Brit. Med. Jour., 1897, vol. ii, p. 1778. Also Medical News, 1901, vol. lxxviii, p. 728.

[2] Medical News, 1902, vol. lxxx, p. 584.

[3] Zeit. f. Hygiene, 1902, Bd. xxxix, p. 283. See also *Lipschutz:* Cent. f. Bakt., 1904, Bd. xxxv, p. 798.

[4] Hyg. Rundschau, 1901, Bd. xi, p. 925.

In disease of the **pancreas,** Gross has used a 0.5 per thousand solution of casein (Grübler) in a 1–1,000 sodium carbonate solution in testing for trypsin in the stool. About 3 c.c. of the stool are rubbed in a mortar with 10 c.c. of a 1–1,000 sodium carbonate solution and filtered. One hundred c.c. of the casein solution and 10 c.c. of the stool filtrate are placed in a flask in the incubator, and a small quantity tested from time to time with a few drops of 1 per cent. acetic acid which produces no precipitate when digestion is complete. The normal time required is about twelve hours. Infants' stools and stools from persons on a meat diet digest much more rapidly than do those from persons on a carbohydrate diet. It has been shown that digestion in this test may be caused also by erepsin, but both erepsin and trypsin disappear from the stool when the pancreas is severely diseased. If an absolute differentiation is desired, a Mett tube (page 293) filled with sheep serum should be employed. Under these circumstances even normal stools may show no trypsin unless the patient is given an active purge.

In **intussusception** of the intestine there may be movements of blood and mucus, and rarely a spontaneous passage of the slough of the intussusceptum from the anus.

In **thrombosis** of the **mesenteric artery,** in chronic congestion of the portal system, such as accompanies cirrhosis of the liver, and in scurvy, there may be large amounts of blood in the stools.

## VI. CHEMISTRY OF THE FÆCES

### TOTAL SOLIDS

In order to determine the amount of dry residue contained in the fæces, the specimen should be mixed with a small quantity of alcohol and heated over a water bath with constant stirring. Small amounts of alcohol should be added to the fluid from time to time, in order to lower the boiling point of the mixture and hasten the evaporation.[1] If a determination of the total nitrogen of the fæces is to be made, the escape of ammonia should be prevented by adding a small quantity of dilute sulphuric acid. Stools containing large amounts of fat dry out with difficulty and it is an advantage to mix them with a previously weighed amount of clean, dry sand, which prevents the formation of coarse lumps and renders the mass more porous so that drying takes place quickly.

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[1] *Poda:* Zeit. f. phys. Chemie., 1898, Bd. xxv, p. 353.

When all the fluid has been driven off from the specimen, it should be placed in an exsiccator over sulphuric acid and the final drying hastened by exhausting the contents of the vessel with a filter pump.

The determination of the solids of the fæces is of comparatively little importance from the diagnostic point of view, and the method here given is of interest chiefly as it is a preliminary to the determination of the total nitrogen and fat content.

### TOTAL NITROGEN

One or two grams of the dried fæces are weighed out in a watch-glass and placed in a Kjeldahl flask with 20 c.c. of sulphuric acid and a few drops of copper sulphate solution. The mixture is heated until the fluid becomes perfectly clear, which usually requires three to four hours. Further details of the method will be found on page 468.

The amount of nitrogen excreted in a fasting condition is from 2 to 4 grams per day. On a mixed diet, the daily excretion may rise to 4 or 5 grams. On a diet composed largely of vegetables the total loss in the fæces may be 9 or 10 grams per day.

In estimating the results of metabolism experiments on patients in order to determine loss or retention of nitrogen or urea in disease, the nitrogen excreted in the fæces should always be taken into consideration, especially as it has been shown that in nephritis, the amount is very likely to be increased by transudation through the intestinal mucosa, thus diminishing the amount of nitrogen excreted in the urine. This phenomenon, however, is not confined to persons the subject of kidney disease, but may be seen in leukæmia, gout, and diabetes.

### ALBUMINOUS SUBSTANCES

The presence of albumin and globulin in the fæces can be demonstrated by making an aqueous extract of the fresh excreta, filtering, and carrying out the ordinary reactions for albumin, such as are given under the subject of albumin in the urine.

If the albumin is removed by coagulation, the presence of albumose and peptone can be demonstrated by the Hofmeister-

Salkowski method as given on page 512. Quantitative tests for these substances are quite unimportant; in fact, no diagnostic conclusions can be drawn even from the qualitative reactions.

## CASEIN

The determination of the presence of casein coagula in the stools is of importance, especially in infants, as it furnishes valuable hints as to the completeness of the digestive processes in the intestinal tract. If coarse flocculi of casein are present in the stools they should be removed with a platinum loop and tested by the addition of a little acetic, or 5 per cent. hydrochloric acid. Casein is easily soluble in either of these reagents. The small whitish masses so often seen in the stools of normal infants are usually composed of masses of fat crystals and bacteria, united by a small amount of mucus. These white particles are found in perfectly normal stools and should not be confused with casein flocculi.

Coarse white flocculi are an evidence of intestinal disturbance, and though usually assumed to be casein are, according to Leiner, pseudonuclein or a closely related body. Leiner[1] suggests as a test for small amounts of casein, the use of a stain composed of acid fuchsin and methyl green. A small quantity of fæcal material is spread out on a slide and dried. The specimen is then fixed by passing through a flame and stained with a mixture composed of equal parts of a $\frac{3}{4}$ per cent. solution of acid fuchsin and methyl green in 50 per cent. of alcohol. This staining mixture should be diluted ten times with water before use.

The preparation is submitted to the action of the stain for fifteen minutes, at the end of which time it is placed in distilled water for an hour. Casein and paracasein are stained blue or a violet color, while other substances take a pale green or yellow.

## MUCUS AND NUCLEO-ALBUMIN

The test usually given for mucus, by extracting the fæces with lime-water and precipitation with strong acetic acid, is really a reaction for nucleo-albumin, rather than for mucus. The identification of the nucleo-albumin extracted in this manner depends upon the demonstration of the phosphorus which this substance contains, or isolation of one of the purin bodies. These chemical procedures are much too complicated for clinical work, and the diagnostic results which may be obtained are not of sufficient value to warrant carrying out the process.

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[1] Jahrbuch. f. Kinderheilkunde, 1899, Bd. l, p. 321.

The same is true of the chemical tests for mucus. It is better to rely upon the results of macroscopical and microscopical tests than upon chemical methods of extraction.

### THE DECOMPOSITION PRODUCTS OF THE PROTEIDS

**Leucin** and **tyrosin** can be demonstrated in the fæces by extraction of the dry residue by strong hot alcohol. The alcoholic solution is evaporated, and the residue dissolved in water and precipitated with lead subacetate. The excess of the subacetate is removed with hydrogen sulphide and the filtrate is evaporated to dryness. Leucin and tyrosin crystallize out and can be further tested by the chemical reactions given on page 457. No facts of diagnostic value are known concerning their presence in the fæces.

**Indol, skatol,** and bodies of the **phenol** and aromatic acid group can also be demonstrated in the fæces. Indol and skatol can be separated from the fæces by diluting the latter with a considerable bulk of water, acidulating with phosphoric acid, and then distilling off a third of the fluid. The distillate contains volatile fatty acids, indol, skatol, and phenol. The distillate is rendered alkaline with sodium carbonate and distilled a second time, the fatty acids remaining as a sodium compound. The second distillate is rendered strongly alkaline with sodium hydrate and again distilled, when indol and skatol pass over, the phenol being retained in the alkaline fluid. Indol and skatol can then be determined in the distillate by the tests given on pages 443 and 444.

Both substances can be demonstrated, if present in the distillate, by the para-dimethyl-amido-benzaldehyde reaction of Ehrlich. Skatol is usually present in the fæces of normal individuals in traces only, and the red color produced on adding the aldehyde reagent (see page 444) and heating if necessary, is in general due to indol. In one case of anæmia and in one of diabetic coma, Herter found skatol only and no indol in the distillate from the fæces. A method of separation of indol and skatol and the colorimetric determination of each component has recently been worked out by Herter,[1] and though somewhat complicated promises to be of much value in the study of intestinal putrefaction. Herter has shown that the amounts of indol in the fæces and of indoxyl in the urine run a roughly parallel course. The skatol reaction of the urine and also the Ehrlich aldehyde reaction when applied directly to that fluid are increased by the oral administration of skatol. The direct estimation of indol in the fæces as suggested by Schmidt and others[2] has been shown to be questionable, as the Ehrlich reaction is probably also given by urobilinogen.[3]

### BLOOD IN THE FÆCES

Blood or blood pigment may appear in the fæces in large quantities from a hæmorrhage from the opening of a vessel of considerable size, in which case the altered blood forms a thick, tarry, blackish mass; or it may appear in small amounts evenly distributed throughout the fæcal mass and not recognizable by the unaided eye. The most frequent condition connected with the finding of very minute traces of blood in the feces is carcinoma of the stomach, or duodenum, or caput coli. Traces are occasionally found in cases of chronic gastritis. As a rule, in any hæmorrhage which occurs above the sigmoid flexure the morphological elements of the blood are so altered by the action of the intestinal juices that they cannot be recognized by the microscope. Dark particles may be seen which are composed of alkaline hæmatin formed by the alkaline digestive juices, but they are not characteristic. The amount of change undergone depends somewhat upon the speed with which the intestinal contents pass through the bowel and also upon their consistency. In a firm fæcal mass the blood may be protected from change by a covering of mucus. In hæmorrhages near the rectum, bright, unaltered blood, in which the red corpuscles may be identified by the microscope, may be found, usually on the surface of the fæcal mass.

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[1] *Herter* and *Foster:* Jour. Biol. Chem., vol. i, 1906, p. 257; ibid., vol. ii, 1906, p. 267; *Herter:* ibid., p. 8.

[2] *Schmidt:* Münch. med. Woch., 1903, p. 721; *Baumstark:* ibid., p. 722; *Ury:* Deut. med. Woch., 1904, p. 700; *Moraczewski:* Zent. f. innere Med., Bd. xxv., 1904, p. 593.

[3] *Bauer:* Zent. f. innere Med., Bd. xxvi, 1905, p. 833.

Very few diagnostic points can be drawn from examination of the stool as to the locality of the bleeding area. Ulcers of the mucosa, invagination of a portion of the gut, stenoses, and new growths, may all produce intestinal hæmorrhages, the site of which can only be determined by physical signs or clinical symptoms.

1. **Teichmann's test,** preferably with Strzyzowski's modification (page 18), may be used to identify blood when found in the stools in macroscopic amounts; but when it is present in very small quantities, as may occur in ulceration or in the bleeding from new growths of the stomach or intestinal tract, it is necessary to use more delicate methods.

2. **Weber's Test for Blood Pigment.**[1]—The most satisfactory test depends upon the extraction of the pigment with acidulated ether and its identification by means of the spectroscope. About 10 c.c. of the fæces are stirred up in a little water in a large test tube and shaken out with *neutral* ether to remove the fat. One c.c. of strong acetic acid is then added, then one-third of the bulk of ether, and the whole thoroughly shaken. In a few minutes the ether separates as a clear layer in the upper part of the tube, and can be poured or pipetted off. The clear brownish solution is then examined with a direct vision spectroscope for the spectrum of acid hæmatin. Generally, only the band in the red is visible, the other bands being obscured by the general absorption of the blue and green end of the spectrum by hydrobilirubin and other pigments. Inasmuch as chlorophyl also gives an absorption band in the red end of the spectrum, which, though not coincident with the band due to hæmatin, is yet so near as to lead readily to error, the test should be completed by adding some sodium hydrate, alcohol and ammonium sulphide to the mixture and thus producing the characteristic two-band spectrum of hæmochromogen. Chlorophyl does not give this spectrum.

In a certain number of cases the hæmochromogen reaction may be obtained directly by treating the fæces with strong alkali, pyridin, and ammonium sulphide, as recommended by Donogány, but for small quantities of blood, Weber's method is preferable. If a spectroscope is not at hand, the blood pigment may be identified by adding ten drops of a freshly prepared solution of resin of guaiac and twenty to thirty drops of ozonized turpentine to the ethereal extract of the stool. If blood is present, the ether assumes a purple color which can be extracted by shaking up with chloroform and water.

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[1] Berl. klin. Woch., 1893, p. 441.

Instead of using tincture of guaiac to produce the color reaction, an extract of Barbados aloin (see page 12) in dilute alcohol may be employed. The reaction is slightly more sensitive than with guaiac.

3. **Benzidin Test for Blood.**[1]—A few granules of benzidin are dissolved in 2 c.c. of glacial acetic acid. A small fragment of the stool is mixed with 2 c.c. of water and boiled in a test tube. Ten drops of the benzidin-acetic acid solution and 3 c.c. of 3 per cent. hydrogen peroxide are mixed in a test tube and a few drops of the cooked emulsion of feces are added. A greenish or bluish color shows the presence of blood. The ethereal extract of the previous test may also be used, adding the benzidin, acetic acid, and peroxide.

This reaction is extremely sensitive and care should be taken to see that the patient is not eating meat. The benzidin may be dried on a paper, as suggested by Einhorn, and the boiled stool mixed with a little peroxide and dropped on the paper. The blue color should appear within two minutes.

### BILE PIGMENTS

The simplest method of testing the fæces for bile pigments is with a concentrated solution of mercuric chloride.[2] A small fragment of the fresh fæces is rubbed up in a mortar or a small glass dish with a few c.c. of saturated aqueous solution of mercuric chloride, and the mixture is allowed to stand for several hours. The portions containing hydrobilirubin take a deep red color, while the fragments colored with bilirubin assume a greenish tone. The color changes are due to the formation of a reddish compound of hydrobilirubin and the bichloride, and to the oxidation of bilirubin into green biliverdin.

The absorption spectrum of hydrobilirubin also serves to identify this substance. It shows a single band between *b* and *F*. In strongly alkaline solutions, the bands extend slightly to the red side of *b*. It is impossible to see these bands if cholecyanin or blood pigment is present.

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[1] *O.* and *R. Adler:* Zeit. f. physiol. Chemie, xli, 1904, p. 59.

[2] *Schmidt:* Verhand. d. Cong. f. inn. Med., 1895, Bd. xiii, p. 320.

Cholecyanin can be recognized by adding ammoniacal zinc chloride solution to an alcoholic extract of the fæces and examining the mixture with a spectroscope. Cholecyanin shows, besides a band approximately corresponding to that of hydrobilirubin, two other bands on either side of *D*.

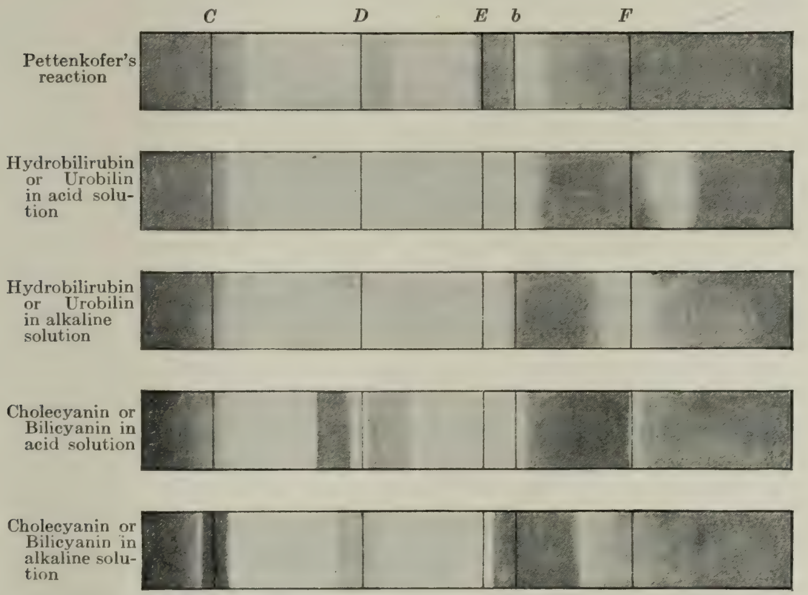
C
D
E
b
F
Pettenkofer's reaction
Hydrobilirubin or Urobilin in acid solution
Hydrobilirubin or Urobilin in alkaline solution
Cholecyanin or Bilicyanin in acid solution
Cholecyanin or Bilicyanin in alkaline solution

FIG. 77.—ABSORPTION SPECTRA OF PETTENKOFER'S REACTION AND OF BILE PIGMENTS.

The diagnostic value of these tests is chiefly in the study of the fæces in children. Bilirubin is present in meconium, and in the stools of nursing children, while occasionally in acute diarrhœas it is possible to find crystals of bilirubin—hæmatoidin—in the fluid fæces. In the dyspeptic diarrhœas of infants, the stools assume a green color due to biliverdin. Clay-colored stools show either that hydrobilirubin is absent or that it exists in the form of leucohydrobilirubin. The presence of leucohydrobilirubin can be demonstrated in some colorless stools by extracting the leucohydrobilirubin with alcohol, adding tincture of iodine or chloride of zinc and ammonia, and submitting the mixture to spectroscopic examination for hydrobilirubin. If the latter is not found, the pale color of the stools is an evidence of complete obstruction to the entrance of bile into the intestinal tract. It is important to remember that stools containing large amounts of fat may be very pale in color, although hydrobilirubin is present in abundance.

## FAT IN THE FÆCES

Only a very moderate amount of the fat taken in escapes from the body in the fæces under normal conditions. A portion of this fat is derived from the mucous membrane of the intestine, from which is excreted, in starving people,[1] about 1 gram of fat in twenty-four hours, or about 25 per cent. of the total solid residue. Fifty per cent. of this is in the form of neutral fats and cholesterin, 40 per cent. fatty acids, 10 per cent. as soaps.

Healthy persons on an approximately fat free diet may excrete as high as 6 grams of fat per day.

Adults on a milk diet excrete 1.5 to 7.5 grams per day, depending on the quantity of fluid taken and differences in individuals.

On a mixed diet containing about 5 grams of fat, about 57 per cent. reappears in the fæces; on a diet containing 43 grams of fat, 11 per cent. is lost in the fæces; while on a diet containing 80 grams of fat, 6 grams are lost. The assimilation limit of fat is about 350 grams per day, if it is taken in an easily digested form such as butter. There are marked individual differences in persons as to their digestive capacity for fats, and in the same individual there are often considerable fluctuations in the daily fat excretion. C. v. Noorden,[2] for example, has noted a daily variation in the fat excretion between 0.5 and 4.5 grams, when the amount of butter given per day was approximately constant.

In pathological conditions, also, marked variations in the excretion of fat are noticed. In infants, who normally absorb some 80 per cent. of the fat in the food, a slight intestinal disturbance often greatly diminishes the absorption. An increase of 20 per cent. in the fat excreted has been noted in infants during dentition without definite intestinal symptoms. In catarrhal gastroenteritis with so-called infantile fatty diarrhœa, 50 per cent. of the fat taken in may be excreted. In icterus neonatorum the fat may make up 40 per cent. of the dried residue as against the normal proportion of 20 per cent.

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[1] *Fr. Müller:* Virchow's Archiv, 1893, Bd. cxxxi, Suppl. Heft, p. 1.

[2] Lehrbuch der Pathologie des Stoffwechsels, Berlin, 1893.

In adults the absorption of fat is not much influenced by disturbance of the gastric digestion or by a moderate catarrhal enteritis. If the bile, however, does not freely enter the intestine, the fat absorption becomes very incomplete, the excretion rising from the normal of 20 per cent. of the dried residue to 30, 40, or even 50 per cent. Persons with biliary obstruction absorb only about 25 per cent. of the fat taken in, while a normal person absorbs on an average 95 per cent.[1]

Diminution or absence of the pancreatic secretion may or may not influence fat absorption. Some observers have not been able to determine any variation from the normal. Deucher,[2] however, in two cases suffering from pancreatic disease, found that 52 and 83 per cent. respectively of the fat taken in remained unabsorbed.

Stools containing an excess of fat have occasionally been noted in persons suffering from severe intestinal catarrh, from carcinoma of the stomach or intestine, and in intestinal tuberculosis, but not often enough to warrant any diagnostic conclusions. In leukæmia the stools may contain large amounts of fat. A few cases have been reported[3] in which no marked intestinal disease could be made out, yet the stools contained large amounts of fat. The administration of pancreatin in large doses did not improve the fat absorption, so the phenomenon was due in all probability to a functional disturbance of intestinal digestion and not to a lesion of the pancreas.

The blocking of the pancreatic duct by calculi has occasionally, though not always, been accompanied by a large loss of fat in the stools, but the condition is suggestive of a pancreatic lesion only in the *absence* of jaundice.[4]

Animal experiments show quite constantly a high loss of fat when the pancreatic excretion is completely suppressed; but easily digested, emulsified fat, for example, in the form of milk, is much more completely absorbed than oils. In some cases of stone in the pancreatic duct, or atrophy of the glandular substance, Müller [1] has shown that the splitting of the fats in the intestine into acids and glycerin is very incomplete; but this phenomenon is not constant and can not be used as diagnostic of pancreatic lesions. The splitting of the fat into fatty acids in some cases is nearly as complete as in normal persons, an evidence that the fat-splitting ferment of the stomach plays an important part in the digestion of this substance.

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[1] *Schmidt* u. *Strasburger:* Die Faeces des Menschen, Berlin, 1901, p. 153.

[2] Correspondenzbl. f. Schweizer Aerzte, 1898, pp. 321 and 361.

[3] See *Salomon:* Ueber Fettstühle, Verh. d. Kong. f. inner. Med., 1902, p. 244.

[4] For further details see *Lazarus:* Beit. z. Path. u. Therap. d. Pancreas-Erkrankungen, Berlin, 1904, p. 171.

**Quantitative Tests.**—The diagnostic value of quantitative analyses to determine the amount of fat in the stools is very slight. As a preliminary, the amount of fat in the food must first be determined, and the fæces must be carefully delimited every twenty-four hours by the administration of 0.3 of a gram of carmine or a charcoal tablet. If diarrhœa is present, it is impossible to make accurate determinations, and the fæces should be collected for a considerable period; for example, three or four days. After mixing, an aliquot part is used for the analysis. The fat intake during the same period must be carefully determined.

An additional complication arises from the fact that the normal absorption varies within considerable limits. In children the amount is fairly constant at 20 per cent. of the dried residue, but in adults it may be from 12 to 18 per cent., when the food taken in leaves but little residue, and from 25 to 30 per cent. on an abundant mixed diet. These figures are only the relation between the fat and the dried residue, and in order to obtain a basis for the total absorption in normal persons, experiments have been made on healthy people, with the result that, as stated above (p. 333), 5 grams may be considered as an average daily loss, 95 per cent. of the fat taken in being absorbed.

**Technique.**—The total fat is determined by extracting a weighed amount of dry fæces with ether.

A considerable quantity of dried and finely pulverized fæces is mixed in a porcelain dish with a small quantity of 1 per cent. hydrochloric acid in alcohol, and evaporated to dryness on a water bath. The addition of the acid alcohol is to split the soaps which are present in the fæces into free fatty acid and neutral salts, in order that the fatty acid may be extracted by the ether, the soaps not being soluble in that menstruum.

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[1] Zeit. f. klin. Med., Bd. xii, 1887, p. 45.

About 5 grams of the dried and pulverized mass are weighed off and placed in a paper cylinder and extracted with ether for three days in a Soxhlet apparatus (Fig. 78). The ethereal extract is evaporated to dryness by a gentle heat and the residue dissolved in *anhydrous* ether and filtered into a weighing bottle. The filter should be washed with some ether and the washings added to the first filtrate. The ether is then evaporated by a gentle heat and the weight of the vessel and its contents determined on a balance. This total ethereal extract contains neutral fats, fatty acids, cholesterin, coprosterin, lecithin, cholalic acid, and other substances, but the approximate result is close enough for clinical purposes.

Rosenfeld has recommended the use of chloroform for the extraction, which has the advantage that the time required is only six hours, as compared with the three days' extraction by the ether method. Petroleum benzine may also be used to extract the fat.

A separate determination of the neutral fats, fatty acids, and soaps, may be made as follows:

(*a*) The dried fæces which have not been treated with acid are exhausted with ether. The extract so obtained contains the neutral fat and the fatty acids.

(*b*) The portion of the fæces remaining in the paper cylinder is then moistened with hydrochloric acid and alcohol, dried, and extracted a second time with ether, and the extract weighed. This gives the fatty acids split off from the soaps by the acid.

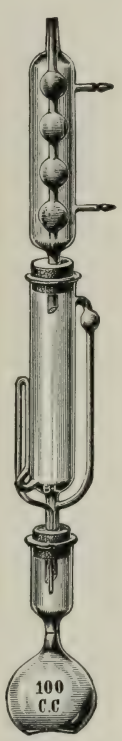

FIG. 78.—SOXHLET FAT EXTRACTION APPARATUS.

(*c*) The volatile fatty acids can be removed from the first extract by washing with some water, in which they are soluble. The residue of fat which is left after washing is then dried and weighed. The difference gives the volatile fatty acids which are dissolved in the wash water.

The residue obtained by (*a*) is then dissolved in a mixture of equal parts of alcohol and ether, and titrated with alcoholic decinormal potassium hydrate, using phenolphthalein as an indicator. A permanent faint pink is the end reaction. One c.c.

of decinormal potassium hydrate solution is equivalent to 0.0284 grams of stearic acid. The number of c.c. of alkali used are multiplied by this factor and the product is the fatty acid extracted from the fæces. The residue is the neutral fat, cholesterin, coprosterin, and lecithin. The cholesterin and coprosterin may be separated and weighed by the procedure given in the following section. These special chemical methods are of no clinical interest.

### CHOLESTERIN

This substance or a body closely related to it, coprosterin, is present in the fæces and may be completely extracted by ether. Cholesterin is found in the fæces of children and adults on a milk diet, while in adults on a mixed diet the cholesterin is reduced to coprosterin by the action of the intestinal bacteria.

Qualitative tests for cholesterin or coprosterin can be carried out with the ethereal extract of the fæces obtained for the purpose of estimating the fat excretion. (See page 337.)

The fat is separated from the cholesterin and coprosterin by heating the residue for half an hour on a water bath with alcoholic potassium hydrate. The alcohol is evaporated, the residue is extracted with ether or diluted with water and shaken out with ether, and the ethereal extracts collected and evaporated. The cholesterin and coprosterin in this residue may be separated for the purpose of identification by extraction with cold alcohol, in which cholesterin is not soluble. If the ethereal solution is evaporated, the cholesterin separates out in characteristic rhomboid plates, while the coprosterin forms long curved needles.

### LECITHIN

This substance can be found in very small quantities in the fæces. It is derived from the lecithin of the food. It is extracted by ether from the fæces, and when the extract is saponified, it breaks up into fatty acid, neurin, and glycero-phosphoric acid. Qualitative or quantitative tests are of no diagnostic value.

### CARBOHYDRATES

**Glucose** and **lactose** have occasionally been found in the stools of persons suffering from intestinal disorders, but not under normal conditions. The presence of sugar is merely an evidence of insufficient absorption by the intestinal mucosa.

**Starch** is found in increased amount in the fæces in intestinal catarrh and in what has been termed fermentative intestinal dyspepsia. The qualitative recognition of its presence in the fæces may be made by the microscope or by the Schmidt fermentation test, which has been previously given on page 319. Quantitative determinations are of no practical value.

Complete exclusion of the pancreatic juice from the intestinal lumen does not necessarily interfere with starch digestion.

## GALL-STONES

Gall-stones are occasionally found in the fæces after passage from the gall-bladder, but only after careful washing and sifting of the excreted material. The softer gall-stones often disintegrate and can not be demonstrated in the fæces.

Naunyn has classified gall-stones into the following groups:

1. Pure cholesterin stones with a smooth or granular surface, whitish color, and crystalline structure.
2. Cholesterin stones arranged in layers, the layers often being of alternating dark and light color.
3. Stones with the material arranged in layers, usually varying somewhat in color, but not containing crystalline masses.
4. Mixed gall-stones composed of calcium bilirubinate and cholesterin arranged in layers and colored. The nucleus is often of cholesterin.
5. Stones of pure calcium bilirubinate. These are dark brown in color and very friable. Cholesterin is usually present only in small quantities.

**Chemical Examination of Gall-stones.**—The stone is powdered and extracted with a little hot water to remove the bile acids. The residue is then extracted with a warm mixture of equal parts of alcohol and ether, which dissolves the cholesterin. The residue contains the calcium salts and the bile pigment and inorganic substances insoluble in water.

In order to identify the cholesterin the alcohol-ether solution is evaporated and submitted to the following tests:

1. A few of the rhomboidal crystals of cholesterin are placed on a slide, covered with a cover glass and concentrated sulphuric acid is allowed to run under. The crystals soften at their edges and assume a brilliant carmine red color. If Gram's solution is added, they become blue, red, violet, or green.

2. A small quantity of perfectly dry cholesterin is dissolved in glacial acetic acid and several drops of concentrated sulphuric acid are added. A violet color is produced which passes over rapidly into green. The presence of water interferes with the test.

Bilirubin may be demonstrated in the residue left after the alcohol-ether extraction by adding a small amount of dilute hydrochloric acid to the deposit. If gas is given off, it shows that some calcium carbonate is present. After the acid has acted for a few minutes, the bile pigments are set free from their calcium compounds and may be extracted by chloroform. The chloroform extract is allowed to evaporate and the yellowish residue is tested for bile by the procedures given on page 563

## INTESTINAL CALCULI

Large stone-like masses (coproliths) may be produced by the inspissation of the fæces in the large intestine or in the vermiform appendix. These intestinal concretions may reach a large size, become very hard, and occasionally cause intestinal obstruction.

True intestinal calculi (enteroliths) are much smaller than these large intestinal stones, and resemble the calculi found in the bladder. They consist usually of an organic nucleus composed of vegetable particles, blood clots, etc., on which is deposited layers of intestinal débris and phosphates of the alkaline earths.

The following varieties may be distinguished:

1. Typical intestinal stones, which are round, heavy, very hard, and arranged in concentric layers, and contain some foreign body as a nucleus. These stones are frequently seen in the appendix, as are also the following:

2. Stones which are much lighter, and more friable in consistence, made up chiefly of undigested vegetable fragments encrusted with phosphates. These stones usually show no concentric arrangement nor well-defined nucleus. In this class belong the so-called oatmeal stones, which are occasionally seen after long use of this substance as food.

3. Stones are occasionally seen in persons who have taken continuously large quantities of salol, magnesium, or calcium carbonate.

4. Intestinal gravel, which consists of small hard particles composed of an organic substance with calcium carbonate and magnesium phosphate. Occasionally this gravel is almost entirely vegetable in its nature, and the writer has seen a specimen made up very largely of stone cells derived from pears which the patient had consumed in large quantities.

Stones from the pancreas are very rarely found in the fæces. They are very easily broken up and have a rough surface. They consist chiefly of carbonate and phosphate of lime. For analytical methods see page 610.

# PART IV

# PARASITES

## I. RHIZOPODA

**Amœba dysenteriæ** (*Entamœba histolitica*).—This parasite is made up of a nucleus surrounded by a coarsely granular mass of protoplasm, which contains several vacuoles and often portions of red corpuscles from the blood. The pseudopodia which this animal is capable of protruding are, as a rule, composed chiefly of the hyaline ectosarc of the parasite and not of the granular endosarc. Small encysted forms are occasionally seen. (Fig. 79, upper left portion of cut.)

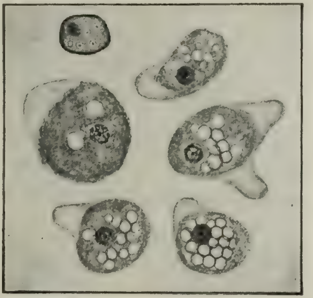

FIG. 79.—AMŒBA DYSENTERIÆ. The two lower parasites are nearly filled with red blood corpuscles. The smaller spherical mass in the upper left corner is an encysted form. (Braun.)

These cells with numerous nuclei are regarded by Grassi as an evidence of growth by multiple division, such as is commonly seen in other types of amœbæ, and may possibly serve to propagate the species outside of the body.

Several other species of amœba (Entamœba coli) have been found in the intestinal contents of those suffering from dysentery, and even in the stools of patients with a simple diarrhœa. They have also been found in the stools of healthy persons, especially after a saline laxative. They are, as a rule, smaller than the amœba dysenteriæ, measuring 10 micra, instead of 20 to 50 micra, as do the pathogenic forms,[1] and do not show a sharp boundary between the ectosarc and endosarc, unless in active motion.

The amœba dysenteriæ has been found in the intestinal mucus, in the walls of ulcers of the colon and rectum, and in a large proportion of the cases of tropical abscesses of the liver.[1]

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[1] For a discussion of the two varieties see *Schaudinn:* Arb. a. d. Kais. Gesundheitsamte, Bd. xix, 1903, p. 547. See also *Casagrandi e Barbagallo:* Annali d'Igiene Sper., vol. vii, 1897, p. 103.

The pus from the liver abscesses is of a yellowish or reddish color and does not, as a rule, contain any pathogenic organisms other than the amœbæ. Generally few leucocytes are present, but much débris, many red blood corpuscles and granular material. The walls of the abscess are covered with shaggy masses containing fibrin and necrotic liver cells. Occasionally the amœbæ can not be found in the pus first aspirated, but appear in large numbers four or five days after drainage has been established by operation.

Whether the amœba stands in direct causal connection with these conditions is still a matter for investigation. At present the trend of opinion seems toward the view that the presence of the amœba is the result of a secondary infection with this organism, and that it is not the primary agent in the disease, especially of the acute forms, which are probably bacillary in origin. The amœbic form of the disease is very wide-spread, occurring in both temperate and tropical regions, though most abundantly in the latter.[2]

The stool or the pus which is to be examined for this parasite must be studied immediately, while still warm, as the parasite easily loses its power of amœboid motion, which is its most characteristic point. The small, grayish masses of mucus should be selected as the most likely site for the parasite. In doubtful cases a long rectoscope tube should be passed and portions of mucus removed from any ulcerated area which may be found and promptly examined on a warm stage. Unless amœboid motion can be detected, a differential diagnosis between the amœba and degenerated cells often found in the stools is difficult. A warm stage during hot summer weather is unnecessary.

In order to make permanent preparations, it is necessary to fix the stool or a fragment of mucus in a sublimate mixture composed of 100 c.c. of an aqueous 1 per cent. mercuric chloride solution, 50 c.c. strong alcohol and 5 drops of glacial acetic acid. After ten minutes the material is transferred to 70 per cent. alcohol containing a few drops of Lugol's solution. It is then washed in water and stained with hæmatoxylin and eosin, dehydrated in alcohol and mounted in balsam.

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[1] *Rogers:* Brit. Med. Jour., vol. ii, 1902, p. 844.

[2] *Councilman* and *Lafleur:* Johns Hopkins Hosp. Reports, vol. ii, 1891, p. 395. *Harris:* Amer. Jour. Med. Sciences, vol. cxv, 1898, p. 384. For a recent review of this subject, with a very complete bibliography, see *Gross,* Deut. Arch. f. klin. Med., Bd. lxxvi, 1903, p. 429.

**Other Forms of Amœbæ.**—Amœbæ have also been found in the mouth, lung cavities, and the urine, but they possess no pathogenic properties and are probably introduced by drinking water or inhalation of dust containing encysted amœbæ. The exact method of infection of the bladder has not been made out.

A parasite resembling the amœbæ and called by Schaudinn, Leydenia geminipara, has been found by v. Leyden in ascites fluid from patients suffering from peritoneal carcinoma, and is considered by him as characteristic of that condition. Other observers are inclined to regard the parasites as merely swollen and altered tissue cells.[1]

## II. FLAGELLATA

**Trichomonas vaginalis.**—The body of the parasite is pear or spindle shape, and measures 15 to 20 micra long and 7 to 12 micra in width. Three or four long flagella are attached to the blunt extremity. The nucleus is near the flagellated end. The body has an undulating membrane attached to one side.

The parasite lives in the vagina in the human subject so long as the reaction is acid. Alkaline irrigation or a change of reaction during menstruation causes the disappearance of the parasite.

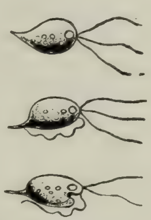

FIG. 80.—TRICHOMONAS VAGINALIS.

This infusorium is probably identical with the Trichomonas intestinalis, which has been frequently found in the mouth, urethra, and intestinal tract of healthy persons, and also in the stools of persons suffering from diarrhœa. The same parasite has been found in the sputum in gangrene of the lung.

There is no reason to suppose that the parasite possesses any pathogenic action in the human subject.

**Lamblia intestinalis.**—This flagellate is a pear-shaped organism, with a large depression on its anterior surface, from the edges of which spring three pairs of cilia. Another pair are attached to the pointed extremity of the parasite. The body measures 10 to 21 micra in length and 5 to 12 in width. The flagella are from 9 to 14 micra in length. Small, oval, encysted forms are found in the fæces, the flagellate forms appearing as a rule only after laxatives have been administered.

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[1] A full consideration of the amœba group may be found in *Braun:* Parasiten des Menschen, third edition, 1903. Also *Doflein:* Die Protozoen als Parasiten u. Krankheitserreger. Zweite Auflage, Jena, 1903.

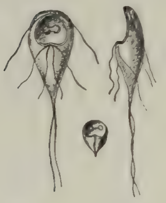

FIG. 81.—LAMBLIA INTESTINALIS.

The parasites are found in the fæces of mice, rats, and other animals. The infectious material is transferred to the human subject by contamination of food with fæces containing the encysted parasites.

**Cercomonas hominis** (*Trichomonas intestinalis*)—Very small parasites with one or more flagella have been found in the stools in a number of intestinal disorders. They have also been found in urine and sputum. The body measures from 10 to 16 micra and occasionally a small nucleus can be made out. They have no pathological importance.

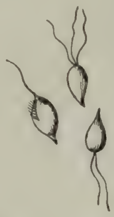

FIG. 82.—CERCOMONAS HOMINIS (*Trichomonas intestinalis*).

## III. INFUSORIA

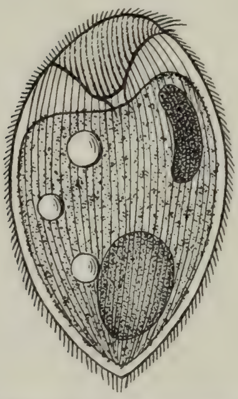

FIG. 83.—BALANTIDIUM COLI. (Leuckart.)

**Balantidium coli.**—This parasite is occasionally found in the lower intestine of man, where it may give rise to dysenteric and diarrhœal symptoms. Some observers have been able to demonstrate the parasite in the intestinal ulcers and in the lymph vessels of the intestinal wall.[1]

The parasite is a small, oval body, 60 to 100 micra long and 50 to 70 micra wide. It is provided with cilia. Two nuclei can be made out, a macronucleus of bean or kidney shape, and a small spherical micronucleus. The blunt end of the parasite is provided with an aperture which is funnel-shaped. The cell body frequently contains fat droplets, starch granules, and bacteria, and occasionally red and white blood corpuscles.

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[1] *Strong* and *Musgrave:* Johns Hopkins Hospital Bulletin, vol. xii, 1901, p. 31. *Ehrnrooth:* Zur Frage der Pathogenität des Balantidium coli, Zeit. f. klin. Med., Bd. xlix, 1903, p. 321 (Bibliography).

## IV. TREMATODA

The trematode group of worms are flat leaf-shaped animals. The Distomida, a family which infects the human subject, are provided with ventrally placed suckers and are hermaphrodite. Bilharzia, in which the sexes are separate, belongs to a slightly different group.

**Fasciola hepatica** (*Distomum hepaticum, Liver fluke*).—This small trematode worm measures about 20 to 30 mm. in length and 8 to 13 mm. in width. It is covered with fine spines, the points being directed backward. The eggs are yellowish brown, and measure 130 to 145 micra in length and 70 to 80 micra in width. They resemble considerably those of Bothriocephalus latus, except that they are much larger.

The parasite commonly inhabits the bile ducts of a number of the herbivora, especially the sheep, beef, and horse. The eggs pass into the intestine through the bile, and the developmental cycle is completed in the body of a small fresh-water mollusk (Limnæa truncatula). The cysts after development in the mollusk may be transferred to aquatic plants and thus infect cattle. Man probably obtains his infection from drinking water.

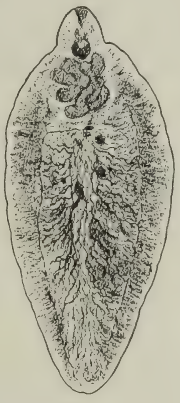

FIG. 84.—FASCIOLA HEPATICA. Magnified 5 diameters.

Human infection is extremely rare, about thirty-two cases having been reported. The usual symptoms are swelling and tenderness of the liver, jaundice, etc. The parasite is often discovered only post mortem, as the clinical picture is not characteristic and the only means of making a positive diagnosis is the finding of the flukes or eggs in the fæces.

**Dicrocœlium lanceatum** (*Distomum lanceolatum*).—This parasite is 8 to 10 mm. in length and 1.5 to 2.5 mm. in width, and has no cuticular spines. The eggs are thick shelled, yellow or brown in color, and measure from 38 to 45 micra in length and 22 to 30 micra in diameter. They contain a flagellate embryo which develops in the snail. Infection in the human subject is rare, about seven cases being reported. It does not cause any characteristic symptoms.

**Opisthorchis felineus** (*Distoma sibiricum*).—This parasite is yellowish red, translucent, and measures 8 to 10 mm. in length and 1.5 to 2 mm. in width. It has been frequently found in the human subject in Siberia, and a few cases have been seen in Germany. A number of fatal cases have shown dilatation of the bile passages with chronic inflammation and atrophy of the liver substance. The eggs are occasionally found in the fæces.

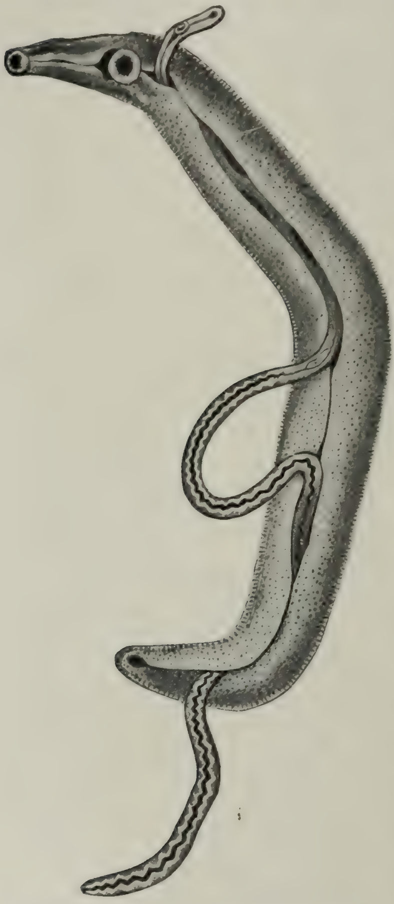

FIG. 85.—MALE AND FEMALE OF BILHARZIA HÆMATOBIA. (LOOSS.)

**Opisthorchis sinensis** (*Distomum spathulatum*).—A very similar worm, of about the same size and color, is abundant in certain districts of Japan and China.[1] The site of the infection is the liver, with resulting atrophy and jaundice. The eggs are frequently found in the intestine and resemble to a certain extent the eggs of Bilharzia hæmatobia. They measure 20 to 35 micra long and 15 to 21 micra broad.

**Paragonimus Westermani** (*Distoma Ringeri ; D. pulmonale*).—This is a small worm of a reddish brown color and of oval shape, measuring 8 to 10 mm. in length and 4 to 6 mm. in width. Infection in the human subject exists chiefly in China, Korea, and Japan, but several cases have been described as occurring in the United States. Hæmoptysis is the most striking symptom of infection by this parasite, and the eggs, which measure 56 by 90 micra, can usually be found in abundance in the sputum. The worms are found in small bronchiectatic cavities or in thick walled cysts.

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[1] See a recent paper by *Inouye :* Arch. f. Verdauungskrankheiten, Bd. ix, 1903, p. 107 (Bibliography).

**Bilharzia hæmatobia** (*Schistosomum hœmatobium*).—The adult forms of this parasite inhabit the portal vein and its branches. They are also found in the vessels of the pelvis, bladder, and rectum. The male measures some 12 to 14 mm. in length and 1 mm. in width, and is provided with a sucker at its anterior end, and a long groove on the ventral surface which contains the female. The latter measures about 20 mm. in length and about 0.25 of a mm. in thickness.

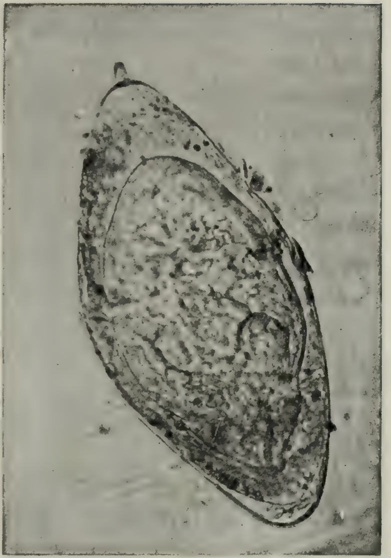
Fig. 86.—Egg of Bilharzia Hæmatobia.

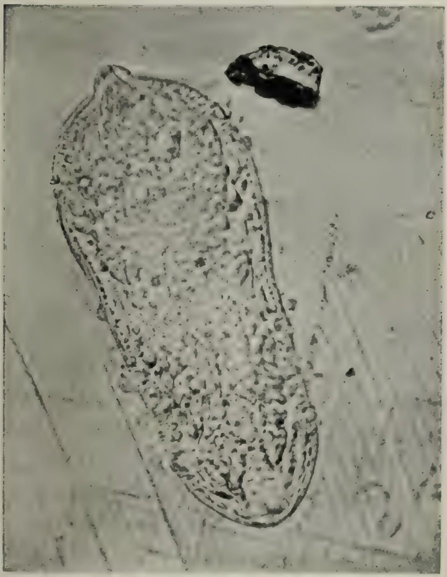
Fig. 87.—Free Swimming Embryo of Bilharzia Hæmatobia.

The eggs of the parasite are spindle shaped and have projecting from the posterior extremity or from the side a small spine. They measure about 0.12 to 0.19 mm. long by 0.05 to 0.07 mm. wide. Inside of the egg is a ciliated embryo which is set free when the eggs come in contact with water.

The method by which man is infected by the parasite is still somewhat doubtful; but it is probably from drinking infected drainage water. Recently it has been claimed that the embryos can enter the circulation through the skin.

The eggs are set free by the female parasite and may very rarely be found in the circulating blood. Usually, however, they enter the bladder and by their presence in the wall of that organ set up a very active cystitis. The urine frequently contains large amounts of blood and pus, while the Bilharzia eggs are abundant. Phosphatic calculi are often found in the bladder in cases of infection by the Bilharzia.

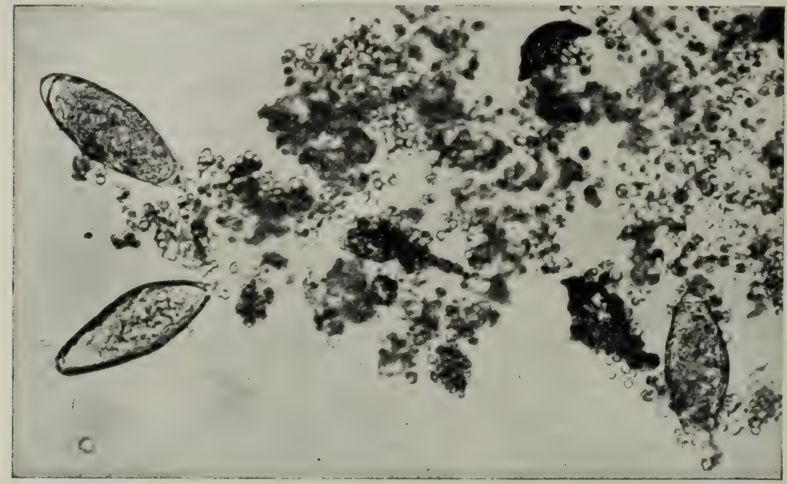

FIG. 88.—OVA OF BILHARZIA HÆMATOBIA IN URINE. The granular masses are blood corpuscles.

The disease is especially prevalent in Egypt, but cases are seen all along the African coast, especially in Algiers and in the south at Natal, Mozambique, and Cape Colony. An isolated center of infection exists in Arabia (Mecca).[1]

## V. CESTODA (TAPEWORMS)

The tapeworms have certain anatomical structures in common which sharply distinguish the group from the nematode worms.

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[1] Further details on the Trematoda may be found in an excellent paper by *Manson*, in A System of Medicine, edited by *Allbutt*, vol. ii, p. 1006. See also *Braun:* Parasiten des Menschen, third edition, 1903; and *Scheube:* Krankheiten d. warmen Länder, Jena, 1903.

They are long, flattened worms without any digestive tract, which are united to form a long row of individuals.

The first member of the series, the head or scolex, is characterized by special structures intended to enable the worm to hold firmly to the intestinal mucosa. Besides these suckers, the head often carries a circlet of small hooks. This first member of the series is to be regarded as the ancestor of the entire worm, for all of the individuals which follow are derived from the head by budding. Each individual of the series is a true hermaphrodite, with both male and female sexual organs, though in the younger members of the chain these organs are not easily seen, while in those which form the free end of the worm, the sexual organs have undergone atrophy and the uterus is altered into a large receptacle for eggs. In some rare species infesting animals the segments are male and female.

The sexual opening is placed along the border of the flattened proglottides or individuals in the Tænias and in the median line in the Bothriocephalus. The uterus usually has a separate opening, either on the lateral border or on the surface. The ventral aspect of the worm is that on which the uterus opens, or the surface to which the uterus most closely approximates.

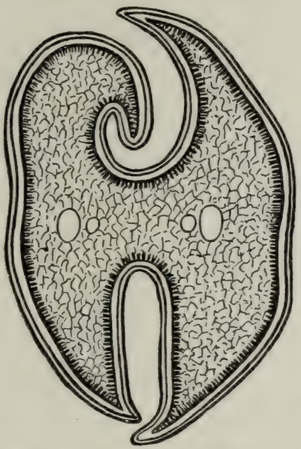
Fig. 89.—Transverse Section of Head of Bothriocephalus Latus. (Moniez.)

The ripe proglottides are capable of independent motion, and in one species, T. saginata, they often crawl out from the anus. Each contains an enormous number of eggs, in which the embryo is already developed, so that in many of the eggs which are found in the fæces the small ring of six hooklets, which is characteristic of the embryo, may easily be seen. The nutrition of the individual proglottides takes place by osmosis with the contents of the intestine and excretion through a water vascular system, the vessels of which pass between the individual proglottides. The injurious effect of the worms on the nutrition of the human host is probably more dependent upon the products of the metabolism of these animals than upon the mere removal of so much food from the intestinal tract.

If the eggs are now taken into the intestinal tract of a suitable host, they bore through the intestinal wall by means of their hooks and are transferred to different portions of the body by means of the blood stream. The liver is naturally frequently invaded because of the number of embryos entering the portal circulation.

When deposited in a suitable tissue the embryo develops into a large cyst, from the walls of which bud out large numbers of scolices which may form secondary cysts. If the contents of one of these cysts is taken into the gastro-intestinal tract of a suitable animal, the wall of the cyst is digested and the scolices contained in it attach themselves to the intestinal mucosa and begin to form the long chain of proglottides known as a tapeworm or strobila.

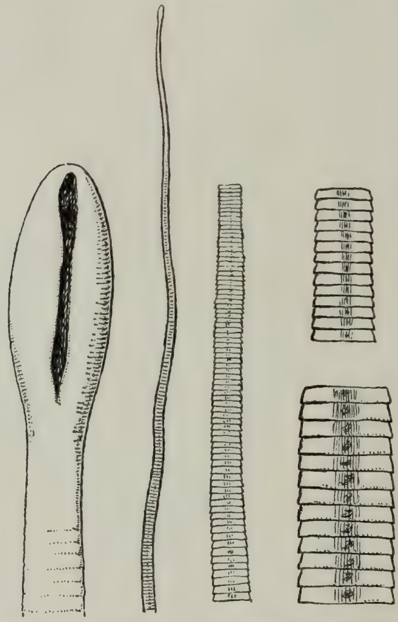

Fig. 90.—Head of Bothriocephalus Latus, much magnified. To the right the head, neck, and proglottides of the same worm about natural size.

**Bothriocephalus latus** (*Dibothriocephalus latus*).—This tapeworm is composed of between three and four thousand individuals, which are ordinarily much broader than long, except near the end of the worm, where they become more nearly square. The oldest proglottides may be longer than they are broad. The length of the worm is from two to nine meters. The head is oval, two or three millimeters long, and has on each side a deep groove which corresponds to the suckers of the other tapeworms (Figs. 89 and 90). The head is not armed with hooks.

The individual segments are of a grayish yellow color. The genital opening is in the middle of the segment and is usually surrounded by a circle of pigment. The uterus shows a number of comparatively simple convolutions which lie near the center of the proglottis and are most easily seen if the worm is soaked in water. (Fig. 91.)

The eggs measure 45 by 68 to 71 micra. They are of a brown color and occasionally a small cap or lid can be demonstrated at one end of the egg. The shell is thin and highly refractile. (See Fig. 92.) The germ cells are often segmented and surrounded by a number of large yolk cells. The eggs are contained in the segments of the middle portion of the worm, the terminal proglottides often containing no eggs. The final development of the eggs takes place in water, and after a number of weeks a small free-swimming embryo is formed, which is covered with cilia. The lid of the egg opens and the embryos pass out of their ciliated shells, sink to the bottom of the water and creep about. The method of infection of the intermediate host is unknown, though the scolices have been found in the intestinal wall, liver, spleen and muscles of various fresh-water fishes, such as the pike, perch, salmon, and carp. When imperfectly cooked fish is eaten, the scolex is set free by the action of the digestive juices and the worm develops in the intestinal tract. The growth is very rapid, and experimental infections in human beings have shown that thirty to thirty-two well-formed proglottides can be developed per day. Eggs appear in the stools twenty-four days after infection of the human being, and the passage of a 22 cm. fragment has been noted twenty-one days after infection.

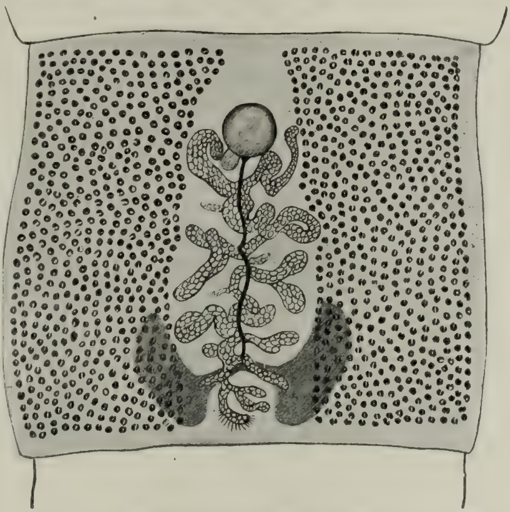

FIG. 91.—PROGLOTTIS OF BOTHRIOCEPHALUS LATUS, SHOWING BRANCHING UTERUS FILLED WITH OVA. (Braun.)

The Bothriocephalus latus is a frequent parasite of the human race in certain localities. It is also seen in dogs, cats, and foxes. The regions in which the population especially suffers from this parasite are the lake districts of Switzerland and the Baltic provinces of Russia. From the first center the infection has spread through France and Italy, and from the Baltic regions to St. Petersburg, Finland, Sweden, Poland, and the coast districts of Germany. Cases are rare along the North Sea and in Holland, Belgium, and northern France. In Turkestan and Japan, Bothriocephalus is one of the most frequent parasites. It is also seen in South Africa. In America a number of cases have been seen, most, if not all, of which were in immigrants.

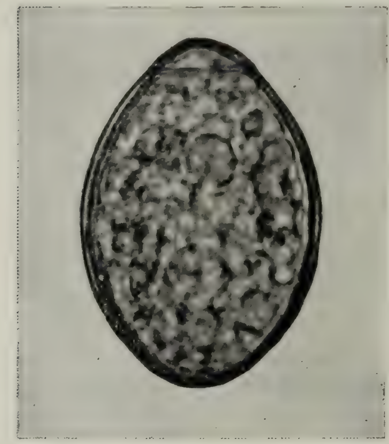

Fig. 92.—Egg of Bothriocephalus Latus, Showing Lid and Yolk Granules. From a photograph by the author.

A considerable number of the population in certain districts is infected by these tapeworms. For example, in Denmark some 10 per cent. of the people carry these parasites; in St. Petersburg about 8 per cent., and in Moscow about 9 per cent. In Switzerland 10 to 20 per cent. of the population living along the banks of the lakes are infected by these worms. In Geneva the proportion was 10 per cent., but is now reduced to 1 per cent. by hygienic measures.

The writer's own experience has been confined to six of these parasites, all of which were obtained from Polish immigrants from the Baltic coast districts. In all these cases only a single worm was obtained from each person, a marked anæmia being noted in one case only. Cases have been reported, however, where a large series of worms have been obtained. For example, Askanazy [1] noted in a case of severe Bothriocephalus latus anæmia a mass of worms from which he was able to isolate 67 heads. Boettcher [2] found at autopsy 100 individuals; Roux [3] found 90; and Heller [4]

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[1] Zeit. f. klin. Med., Bd. xxvii, 1895, p. 490.

[2] Cited by *Leuckart*. English Translation, p. 715.

[3] Correspondenzbl. f. Schweizer Aerzte, 1887, p. 488.

[4] Tageblatt 59te Versamml. Deutscher Naturf. u. Aerzte in Berlin, No. v, p. 147, 1886.

78. It is interesting to note that in the cases of Roux and Heller no anæmia was noted, showing that the blood changes are not in proportion to the number of worms.

The symptoms which the presence of these worms induce in human beings are often unimportant. In some cases there are moderate gastric and nervous symptoms, and in a certain small proportion of those infected there has been observed a very severe anæmia, resembling very closely pernicious anæmia. A fuller discussion of the subject will be found on page 148.

**Diplogonoporus grandis** (*Krabbea grandis*).—The head of this parasite has not been described. The chain of segments measures some ten meters in length. The anterior segments measure 1.5 mm. transversely, the posterior, 2.5 mm. The proglottides are very short, usually not over 0.5 mm. The genital openings are arranged in pairs on the ventral surface of each proglottis. The egg is brown in color, with a thick shell and measures 50 by 63 micra.

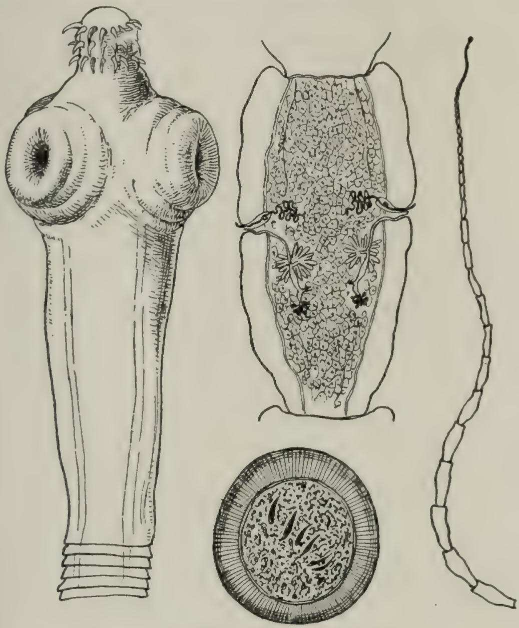

FIG. 93.—DIPYLIDIUM CANINUM. Head, proglottis, and egg considerably magnified. Head and portion of worm about natural size. (Braun.)

The parasite has been found in the human subject only in Japan.

**Dipylidium caninum.**—This worm is a frequent intestinal parasite in the dog, and has been met with in a moderate number of cases in the human subject. It measures from 15 to 35 cm. long and 1.5 to 3 mm. in width. (See Fig. 93.)

The head is small, somewhat rhomboidal in shape, with a projecting rostellum which bears three to four circles of small hooks. The first series of proglottides are short and broad. The ripe members are sausage-shaped, measuring 6 to 7 mm. in length and 2 to 3 mm. in diameter. The genital pores are symmetrically arranged on either side of the proglottis.

The uterus is reticulated and incloses in its meshwork the testicular structures.

The egg is spherical, measuring 43 to 50 micra in diameter.

The intermediary hosts are the dog louse, dog flea, and the human flea. Infection in animals is obtained directly by swallowing infected fleas. The infection in the human subject may arise in a similar manner or may be derived from the cysticercus form, which may be contained in the saliva from the mouth or may be present on the tongue of the dog.

**Hymenolepis nana** (*Tænia nana*).—This small tapeworm averages from 5 to 20 mm. in length, with a maximum breadth of about 0.7 of a mm. The head resembles the Tænia group in that it has four suckers and a row of twenty-four to twenty-eight hooklets, surrounding a retractile proboscis. There are one hundred and fifty to two hundred segments in the mature worm with genital pores on the left. The uterus is not branched, but is single, and in the ripe proglottides is filled with eggs which are oval or round, measuring from 30 to 37 micra in diameter. The structure of the eggs is more complicated than in the Tænia saginata as they have two distinct shells, the inner one presenting at each pole a more or less distinct projection provided with filamentous appendages. The six-hooked embryo can occasionally be made out inside the egg.

The intermediate host is the rat, but it has been shown that this extra cycle can be dispensed with and an autoinfection with the formation of a cysticercus may take place. The eggs of the tapeworm are presumed to be spread by the infection of food by the excrement of mice and rats; secondary infections taking place from person to person, as seems probable from the report of Ransom,[1] where thirty-eight out of one hundred and five cases were inmates of public institutions. A large number of the cases of human infection reported have been from Sicily, where Calandruccio estimates that 10 per cent. of the children harbor this worm. Bilharz has found that it is abundant in Egypt, and cases have been reported in a number of European states, in Japan, Siam, and Brazil, and more recently a number have been

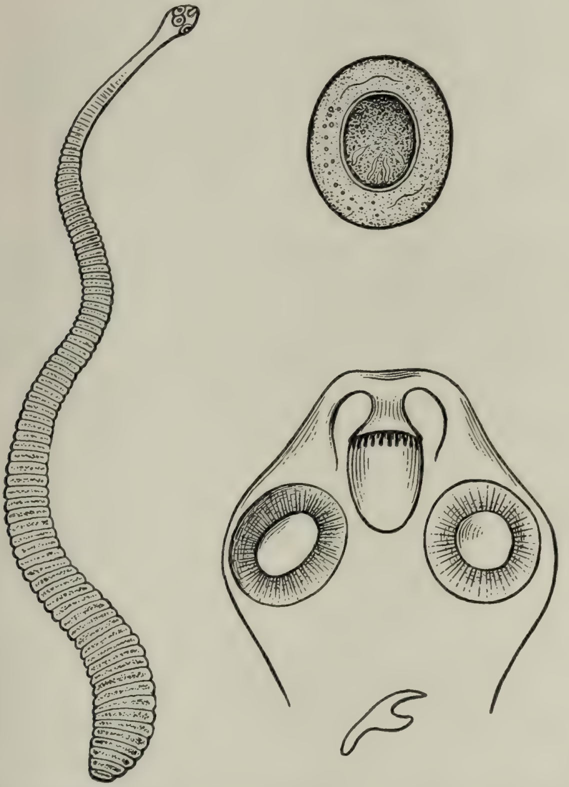

FIG. 94.—HYMENOLEPIS NANA. Parasite to the left, egg, scolex, and hooklet to the right. (Braun.)

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[1] Tapeworms of the Genus Hymenolepis Parasitic in Man. Bulletin 16, Hygienic Laboratory. United States Public Health and Marine Hospital Service, Washington, D. C.

described in the United States, chiefly along the coast of the Gulf of Mexico.[1]

**Hymenolepis diminuta** (*Syn., Tænia diminuta, Tænia flavo-punctata*).—This parasite is a small worm from 30 to 60 cm. long, about 3.5 mm. wide, and is composed of from 600 to 1,000 members. The head is very small, measuring 0.2 to 0.5 mm. in diameter, is oval, and is provided with hooks. The eggs are round or oval, the smaller ones measuring 60 by 70 micra, the larger, 70 by 86 micra. The eggshell is yellowish and shows a faint radial striation. Twelve specimens of this worm have been described in man, three from the United States.[2] It is most frequently met with in Italy. The parasite is frequently found in the intestinal tract of mice and rats. The cysticercus stage has been found in Italy in a meal moth (Asopia farinalis) and in beetles. Other insects can possibly also act as intermediary hosts.[3]

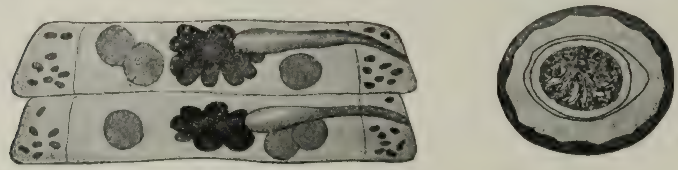

Fig. 95.—Hymenolepis Diminuta and Egg.

**Davainea Madagascariensis.**—This is a rare parasite of some 30 cm. in length, with a scolex carrying four large suckers and a rostellum provided with ninety hooks. The strobila contains some five hundred to seven hundred members. Those forming the posterior half of the worm are filled with eggs. The individual members when mature measure about 2 mm. long and 1.4 mm. broad. The genital pores are all on the same side. The uterus consists of a number of tubes rolled up in small masses which unroll when the eggs develop, the latter being set free in the parenchyma of the segment.

The oncosphere measures about 8 micra in diameter, and is surrounded by two transparent shells from the outer edge of which project two spike-like prolongations.

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[1] For an excellent review of this subject see *Stiles:* New York Medical Journal, vol. lxxviii, 1903, p. 877.

[2] *Packard:* Jour. Amer. Med. Assn., vol. xxxv, 1900, p. 1551.

[3] *Leidy:* Amer. Jour. Med. Sciences, vol. lxxxviii, 1884, p. 110.

The parasite has been found in British Guiana, Africa, and India, but only a moderate number of cases have been reported.

**Tænia solium.**—This worm has an average length of about 2 or 3 meters, with a width of about 8 mm.

The head is knob-shaped and about 1 mm. in diameter. The rostellum is short, often pigmented, and with a double circle of hooks, which average about twenty-six or twenty-eight in number. The suckers are about half a millimeter in diameter. The neck is rather thin and about 5 to 10 mm. long.

The number of proglottides is from eight to nine hundred. They increase very rapidly in size, and about one meter behind the head are square and contain fully developed sexual organs. The old members of the worm which are ready to be cast off are 10 to 12 mm. long and 5 to 6 mm. wide.

The genital openings are placed alternately, first on one side, and then on the other side of the segments. The developed uterus consists of a medium tube from which ten or twelve side tubes extend out on either side. These lateral tubes branch slightly, but not so much as in the Tænia saginata. The eggs are oval; the eggshell proper very thin. The shell of the embryo is thick, radially striated, yellow in color, and measures externally 31 to 36 micra. The embryo (oncosphere) has six hooks and is about 20 micra in diameter.

The mature tapeworm of the Tænia solium is found in the small intestine of man; the head is usually in the first portion of jejunum. The domestic pig acts as intermediate host of the Tænia solium, but the cysticercus is also seen in other mammals, such as the wild pig, sheep, dog, cat, bear, and ape. When the eggs enter the intestinal tract of one of these animals the embryo is set free and enters the wall of the gut, from which it is transported to various portions of the body, where it forms a so-called cysticercus, chiefly in the intermuscular connective tissue. The encysted embryo in the pig is a small, elliptical, white body measuring from 6 to 20 mm. in length and from 5 to 10 mm. in diameter. In the center of the mass a small white spot marks the invaginated head, which can be easily extruded by pressure on the small cyst and can be microscopically recognized as belonging to the Tænia solium.

The cysticercus cellulosa occurs only very rarely in man. It has been seen in the eye, in the brain, and in the other organs. In recent years, owing to the official inspection of meat, the number of cases of infection has been greatly reduced. For example, Virchow, before inspection was begun, found cysticercus in the brain in one autopsy out of thirty-one; while after the introduction of inspection, the number was diminished to one in two

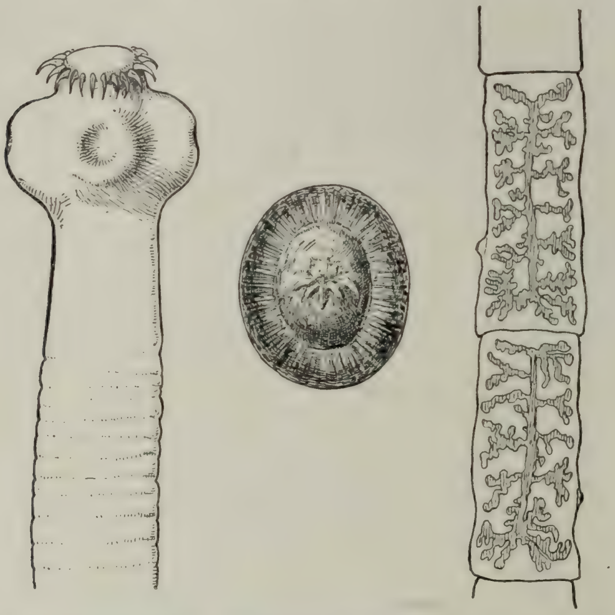

FIG. 96.—SCOLEX, EGG, AND RIPE SEGMENTS OF TÆNIA SOLIUM.

hundred and eighty. The mode of infection in the human subject is a direct transmission of the eggs to the stomach by means of the food or fingers, and possibly by the transmission of ripe segments of the worm from the small intestine past the sphincter of the pylorus into the stomach.

In America, the Tænia solium is exceedingly rare, as the habit of eating uncooked pork is not wide-spread. In some districts in Germany, France and Italy the worm is still occasionally met with, though only in small numbers as compared with the Tænia saginata.

**Tænia saginata** (*T. mediocanellata*).—This species of tapeworm is much more abundant than the Tænia solium, especially in the United States, where rare beef is consumed more frequently than uncooked pork. In Europe, the T. solium is more frequently met with, though even there it forms only a small proportion of the tapeworms of the Tænia group.

The average length of the worm is from 4 to 10 meters, though specimens have been described of 36 meters in length. The breadth of the proglottides varies a good deal, but may be considered to average about 12 to 14 mm., the ripe members being somewhat narrower and measuring about 4 to 7 mm. in width and 16 to 20 in length. The head is roughly cubical and 1 to 2 mm. in diameter. The rostellum contains no hooks. The suckers and rostellum are often pigmented. The uterus sends out a group of from twenty to twenty-five branches, which break up again into a series of smaller tubes. This form of the uterus is important, for it furnishes a differential between this worm and the Tænia solium.

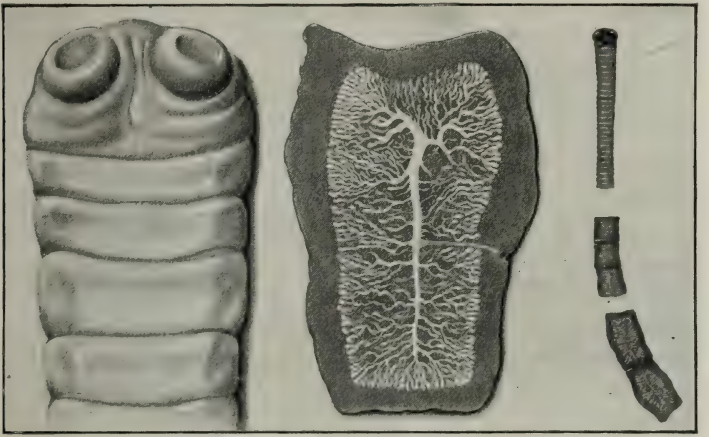
Fig. 97.—Scolex and Segments of Tænia Saginata.

The spherical eggs of the two worms can not be distinguished from each other by the microscope. Those of the Tænia saginata measure, approximately, 30 to 40 micra in length and 20 to 30 in width. The shell is thick, radially striated, and is frequently surrounded by a gelatinous coat.

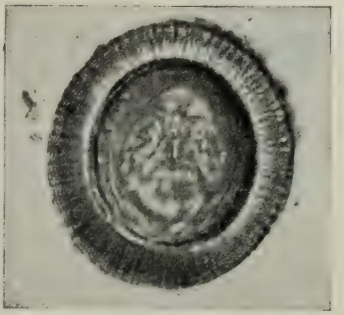
Fig. 98.—Egg of Tænia Saginata.

The extra-human cycle is carried out in the beef, the infection occurring either through the hay used in feeding the animals or by the infection of the grass in the pasture by the segments. The cysticercus form develops in the muscles as small opaque nodules, giving the meat a spotted appearance (measly beef). The cysticercus is very rarely seen in man, three somewhat doubtful cases having been published.

**Tænia echinococcus.**—This small tapeworm normally inhabits the intestinal tract of the dog, fox, jackal, and wolf. The dog is the most important agent in human infection. In Iceland, 28 per cent. of the dogs carry this parasite; in Berlin, 1 per cent.; in Copenhagen, 0.4 per cent.; in Australia, 40 to 50 per cent.; while in America only a small percentage of these animals are infected. The large proportion of infected animals in Australia is apparently due to the fact that the dogs consume the meat of sheep containing the echinococcus cysts.

The adult worm measures 2.5 to 6 mm. in length. The head is 0.3 of a mm. broad, bearing four suckers of a diameter of about 0.13 of a mm. The neck is short. The body consists of three to four members, the one most distant from the head measuring about 2 mm. in length and 0.6 of a mm. in width. The genital pores alternate. The head bears a rostellum carrying a double circle of from twenty-eight to fifty hooks, which vary in size and form.

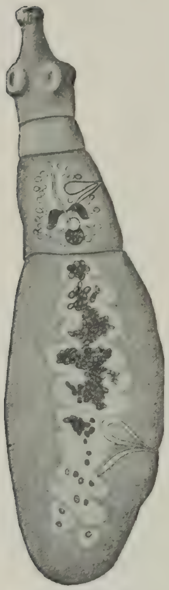

Fig. 99.—Tænia Echinococcus. Magnified 20 diameters. (Braun.)

The uterus is slightly branched and consists merely of a series of irregularly shaped receptacles for the oval eggs, which measure 30 to 36 micra. The shell is thin and radially striated. The adult worm in the small intestine of the dog gives off large numbers of eggs, and the disease is spread by the scattering of the dog's fæces in places from which infectious material can be obtained by children or adults.

Infection has, no doubt, been frequently derived from close contact with house dogs, especially by transfer of the oncospheres, by the dog licking the face or mouth of the child; while in Australia the drinking of water from shallow pools contaminated by dogs' fæces is the common source of the disease.

On the arrival of the embryo in the stomach the shell is digested by the gastric juice, and the six hooked embryo thus freed passes through the intestinal mucosa and is carried by the blood to various portions of the body. Naturally the liver receives the major portion of the infectious material through the portal system. About four weeks after the infection of the body, small cysts about 1 mm. in diameter are developed in the lobular tissue of the organs. These cysts consist of an outer shell formed by the connective tissue of the host and an inner solid body about 0.5 mm. in diameter, which represents the young parasite. The six hooks of the embryo are discarded and the cysticercus consists of an outer transparent shell, the so-called cuticle which is 20 to 50 micra in thickness, and a granular contents. At the end of eight weeks, the cysticercus measures 2 mm., the cuticle thickens, and its inner surface is covered by a thin germinal layer which is at first solid and occupies the entire space inside the cuticle. A cavity soon develops, containing a clear watery fluid. The parasite continues to grow, the outer or cuticular layer becomes laminated, while the germinal layer is composed of a sort of granulation tissue containing a few capillaries and many small cells on the periphery and larger granular cells on the inner surface.

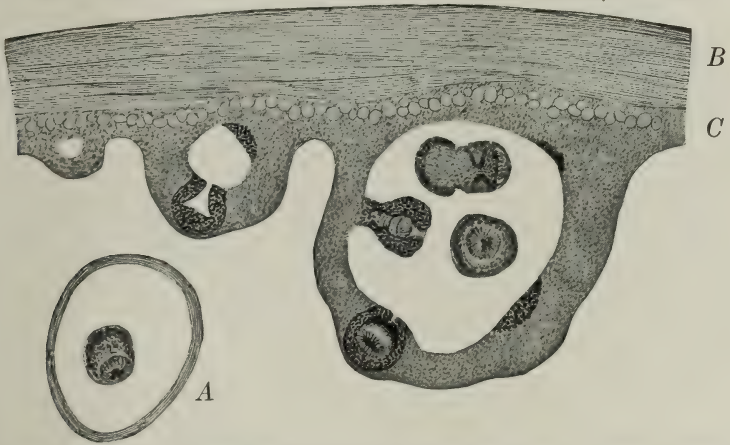

FIG. 100.—DIAGRAM TO SHOW DEVELOPMENT OF THE SCOLICES OF TÆNIA ECHINOCOCCUS AND THE FORMATION OF DAUGHTER CYSTS. *A,* Free Daughter Cyst; *B,* Laminated Cuticle; *C,* Germinal Layer.

Later, a protuberance arises from the germinal layer which projects into the cavity of the cyst and develops into a small capsule within which the scolices of the tapeworm are formed.

The further details of the process are not definitely settled. Leuckart thinks that a diverticulum is formed, which extends into the cavity of the cyst, that the head of the embryo is formed at the base, and that the diverticulum thus formed invaginates and forms the scolex. Moniez,[1] however, claims that the head develops inside of the inner capsule as a protuberance from the inner surface, which may afterwards form a diverticulum enveloping the scolex.

A large number of these brood capsules are formed on the inner surface of a large cyst and become free, giving rise to a series of small translucent daughter cysts containing numerous scolices. These free floating daughter cysts cause the peculiar fremitus felt when an echinococcus cyst is palpated.

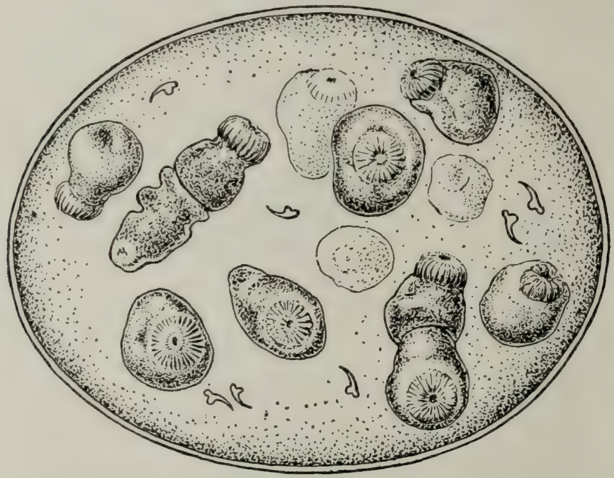

Fig. 101.—Echinococcus Daughter Cyst Removed by Aspiration from a Large Cyst of the Liver. The wall of the cyst is made up of a thin laminated membrane. Inside the cyst are numerous scolices and hooklets, the former in various stages of development. Magnified about 100 diameters.

Degeneration may take place in the cyst with destruction of the daughter cysts and setting free of the hooks and scolices.

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[1] Traité de Parasitologie, Paris, 1896.

The hooks being very resistant to the action of the body fluids, retain their form for years, and if the contents of the cysts are aspirated, the hooks can be found in the fluid.

This fluid is usually clear or of a faint yellow color; either of a neutral reaction or faintly acid; and of a specific gravity of 1.009 to 1.015. It contains about 0.7 per cent. of sodium chloride, traces of glucose, inosite, leucin, cholesterin, and a very small quantity of albumin, which usually does not give rise to a cloud on heating.

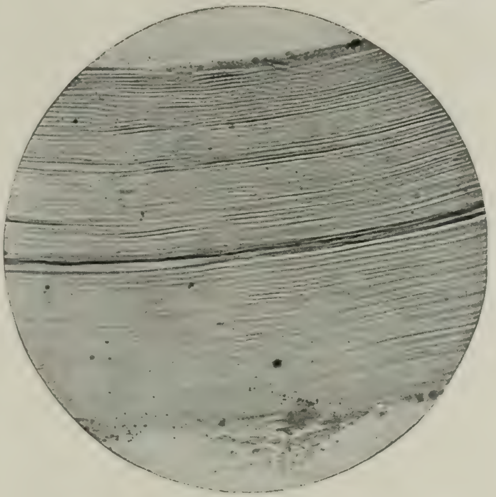

Fig. 102.—Photograph of Laminated Wall from an Echinococcus Cyst.

The laminated membrane is made up of a series of very thin, transparent lamellæ (Fig. 102), and a fragment of it is pathognomonic of an echinococcus cyst.

The scolices (Fig. 103) are small oval or spherical bodies about 0.2 to 0.3 mm. long. The rostellum, which bears the double row of hooklets, may be protruded so that the hooks are visible from the lateral aspect of the scolex; or the rostellum may be so retracted that the hooks can only be seen when the line of sight is directly in the line of the rostellar opening. The suckers are usually quite easily seen as projections from the middle segment.

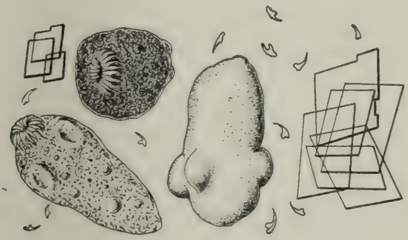

Fig. 103.—Contents of an Echinococcus Cyst showing Scolices, Hooklets, and Cholesterin Crystals.

The cyst may remain single, or the so-called hydatid form may be produced by the production of a large number of small cysts in the walls of the primary cysts. These cysts later become free in the lumen of the larger cyst. The primary cyst thus becomes filled up with daughter cysts, which in turn may contain others. The average diameter of the daughter cysts is from 1 to 2 cm.

The third form of cyst is seen in the human liver, and develops as a series of larger or smaller cysts separated from each other by thick connective tissue trabeculæ. The cyst contents, instead of being thin, are thick and gelatinous, and scolices are difficult to find.

**Tænia Africana.**—This parasite is of comparatively little importance in temperate countries, but has been found in negroes from the Nyassa region of Africa.

The tapeworm measures about 1.3 meters in length. The scolex has no hooks. The proglottides are broad and short. The genital pores alternate irregularly and open on the border of the segments. The uterus has fifteen to twenty-four unbranched receptacles. The embryos are round or oval, with a thick shell which is radially striated. They measure between 30 and 40 micra in diameter.

## VI. NEMATODA

The nematode worms are slender cylindrical animals, tapering slightly at both extremities. The male and female are separate individuals. The eggs of some of the nematodes are capable of developing into what is known as a rhabditic parasite; in other words, the free embryo may develop sexually and form eggs for several generations, its descendants again entering the parasitic stage as true nematode worms.

**Strongyloides intestinalis.**—This parasite is frequently found in Southern China, in the stools of persons suffering from acute diarrhœa. In this country a few cases have been described by Thayer[1] and others.[2] The infection with this parasite is not uncommon in Italy, and scattered cases are reported in Germany. Some have also been seen in the West Indies. The female parasite is a slender worm, about 2 mm. in length by 0.06 mm. in breadth, which may be found in the upper portion of the small intestine, the head being buried in the mucous membrane, or the worm may

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[1] Jour. of Exp. Med., 1901, p. 75.

[2] *Wainwright* and *Nichols:* Medical News, 1904, p. 785. *Brown:* Boston Med. and Surg. Jour., 1903, p. 583. *Strong:* Johns Hopkins Hospital Reports, 1901, vol. x, p. 91. *Price:* Jour. Amer. Med. Ass'n, vol. xli, 1903, pp. 651 and 713. Also *Ward:* ibid., p. 703.

be free in the lumen. No male has been found under these conditions, and the female is supposed to be parthenogenetic. The eggs are not found in the stools unless a very active diarrhœa is present, but develop into so-called rhabditiform embryos, a portion of which may form filariform larvæ, and the remainder may grow into sexually differentiated male and female worms. The rhabditiform embryos measure 0.2 to 0.4 of a mm. in length, and are very actively motile. A few hours after being passed from the anus they may develop into the filariform type, losing all sexual and digestive structures and growing to a length of from 0.4 to 0.7 of a mm. The parasite at the same time becomes more active. The eggs are oval and surrounded by a clear yellow shell, within which can be seen the segmenting embryo. Their dimensions are about 35 by 70 micra.

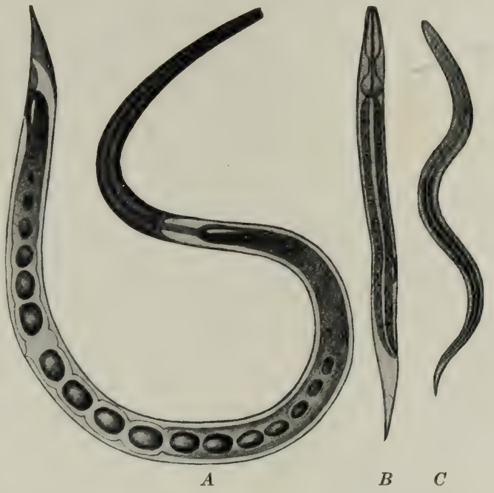

Fig. 104.—Strongyloides Intestinalis. *A*, mature female; *B*, rhabditiform larva; *C*, filariform larva. (Braun.)

**Filaria medinensis.**—The male parasite of this species is not known; the female alone has been observed. The worm may measure 50 to 100 cm. in length and 2 to 3 mm. in diameter. The body is cylindrical and smooth, and the tip of the tail forms a blunt hook, which may possibly act as an anchor to retain the worm in the tissues. The head is rounded, the mouth triangular, very small, and surrounded by six papillæ. In the adult worm, the uterus fills nearly the entire body of the parasite. If the fresh worm is squeezed, a milky fluid is extruded, which when examined under the microscope is seen to contain an enormous number of small filariæ, pointed at one extremity and rounded at the other. These embryos measure 500 to 750 micra in length and 25 to 50 micra in breadth.

The embryos live in muddy water or moist earth for a number of weeks. The intermediate host for the development of the parasite is a small water animal of the genus Cyclops. Whether the infection in the human subject arises from direct entrance of the skin by the parasite or from drinking water containing the Cyclops, is not as yet decided.

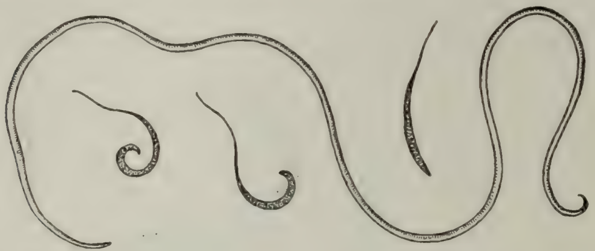

Fig. 105.—Filaria Medinensis. Adult female and filariform embryos. The embryos are more highly magnified than the adult parasite.

The adult worm inhabits the subcutaneous tissue, usually in some part of the lower extremities. An ulcer soon forms about the spot where the parasite enters and the head of the worm can be seen protruding from the hole. By pouring a little water on the ulcer, the worm may be made to discharge some of the embryos contained in the uterus.

The guinea-worm is found in India, Persia, Arabia, and especially in tropical Africa, and some cases have been seen in some parts of Brazil.

The specimen from which the plate (Fig. 105) was drawn was obtained from a negro who had lived on the west coast of Africa.

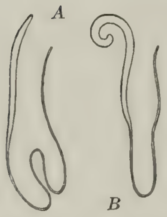

Fig. 106.—Trichocephalus Trichiurus. *A*, female; *B*, male parasite. Natural size.

**Trichocephalus trichiurus** (*T. dispar*).—This is a small worm, the male of which measures about 40 to 45 mm. in length, the female about 45 to 50 mm. The anterior half of the body tapers into a long fine filament. The eggs are oval, and measure 50 to 54 micra long and 23 micra in diameter. They differ sharply from the eggs of the other nematode worms, as on each pole of the egg there is a small projection, which is lighter in color than the rest of the rather dark brown shell. No embryo is visible, only a moderate amount of finely granular yolk. The eggs are very resistant to the action of cold or drying, and the infection of the human host takes place through food, which has been contaminated by the egg, the worms reaching full development about one month after the eggs are swallowed. The mature parasites inhabit the cæcum and the appendix, usually in small numbers, and do not, as a rule, cause any symptoms, although it has been shown[1] that they live upon blood extracted from the intestinal mucous membrane. Occasionally, however, mental symptoms have been produced by the presence of a large number of these parasites.

According to Girard,[2] the Trichocephalus may give rise to lesions of the appendix by penetrating the mucosa with the head. Infection of the mucosal wound by bacteria may then set up acute inflammation. The Trichocephalus is one of the most widely spread and frequently met with intestinal parasites. In Southern Italy nearly 100 per cent. of the population carry these worms. They are abundant throughout Europe, and are met with in England and Ireland among the lower class of the population, and are frequently found in the miners of the coal districts of the United States. They are also seen in the West Indies, the Philippines, and in Southern Asia.

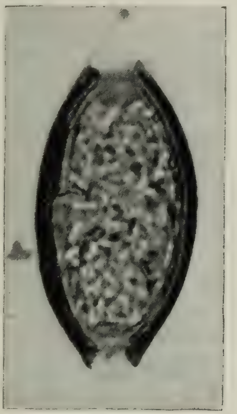

FIG. 107.—EGG OF TRICHOCEPHALUS TRICHIURUS.

The eggs are usually not very abundant in the fæces and the parasites are but rarely seen, as it is exceedingly difficult to expel them by vermifuges.

**Trichina spiralis** (*Trichinella spiralis*).—The adult worms of this parasite inhabit the small intestine of man and of a variety of domestic animals; among the latter are the rat and pig, which together with man are especially susceptible to the infection. The other animals are less likely to act as hosts.

The male worm is from 1.4 to 1.6 mm. long and 0.04 of a mm. thick. The female is 3 to 4 mm. long and 0.06 of a mm. thick.

The rat most often acts as host for the parasite. It is probable that the hog, which is the regular source of infection for man, obtains that infection from the flesh of the rat, as it has been shown that a large proportion of rats carry the Trichina. In America from 2 to 16 per cent. of hogs are infected by the Trichina, varying in different localities.

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[1] *Askanazy:* Deut. Arch. f. klin. Med., Bd. lxxxvii, 1896, p. 104.

[2] Ann. de l'Institut Pasteur, 1901, tome xvi, p. 440.

When the meat of an infected animal is consumed, the encysted embryos of the Trichina are set free in the intestine by the digestive juices and develop to sexual maturity in the duodenum and jejunum. The female, after fecundation, enters the mucous membrane of the intestine, and either remains in the lymph spaces in this situation or enters the lymph nodes or spaces in the mesentery.

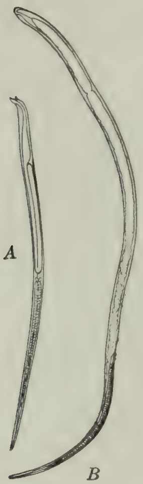

Fig. 108.—Trichina Spiralis. *A*, Adult male worm; *B*, adult female worm. Magnified 25 diameters.

The embryos are given off in large numbers, Leuckart having estimated that each worm furnishes at least fifteen hundred. These embryos measure about 100 micra in length and 6 micra in diameter. They remain of this size until they reach their final position in the muscles of the host. The transfer of the parasites may take place through the lymph channels or the blood stream, and the small embryos enter the muscles of the body through the walls of the small capillaries. The embryo finally enters a muscle fiber, chiefly those near the tendinous insertion.

This infection of the muscles gives rise to acute symptoms with fever and pain in the infected muscles, while very often the infection is accompanied by severe diarrhœa, which may possibly be explained by the injury to the intestinal muscles by the invasion of the small embryos. Small areas of œdema are frequently met with, the most valuable from the diagnostic point of view being an œdema of the eyelids.

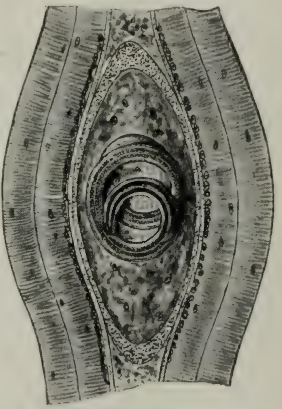

Fig. 109.—Trichina Spiralis Encysted in Muscle.

Two or three weeks after the parasite has entered the muscle it can be seen coiled up and surrounded by degenerated and thickened sarcolemma. In six to nine months the capsule has begun to calcify, and later the parasite itself becomes infiltrated with lime salts. The parasites are apt to be abundant in the deltoid muscle near its insertion, and in the lower portion of the biceps near the tendon. From these situations, small fragments of muscle may be excised and examined with a medium power lens after crushing between two slides.

Occasionally parasites may not be present in sufficiently large numbers to be demonstrated in specimens of muscle which can be excised without injury to the patient, in which case the clinical symptoms—especially the œdema of the eyelids and the marked eosinophilia which is present in a large proportion of cases of trichinosis—will complete the diagnosis. The parasites may confine themselves largely to inaccessible muscles, such as those of the larynx and the diaphragm. It is only rarely possible to find the embryos or adult worms in the stools.

**Eustrongylus gigas.**—The male of this worm is about 40 cm. long and 4 to 6 mm. in diameter. The posterior extremity is provided with a broad bursa, surrounded by papillæ from the

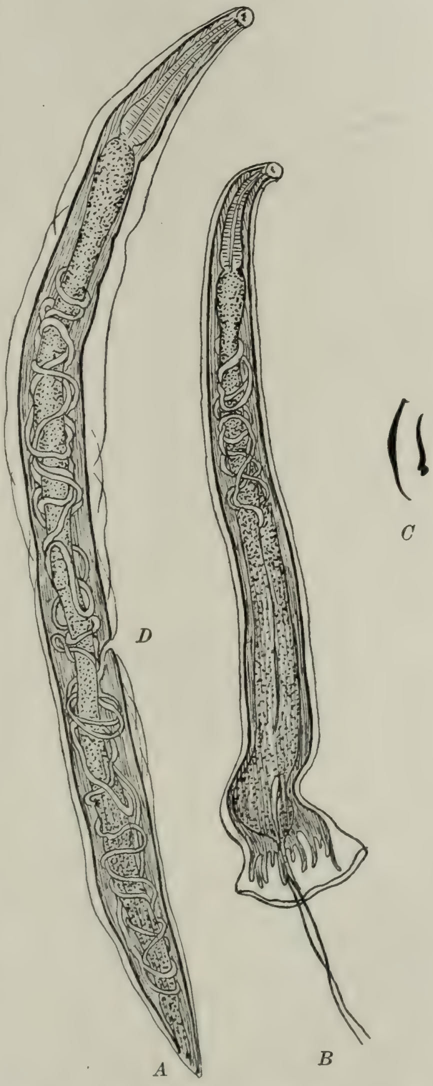

Fig. 110.—Uncinaria Duodenale. *A*, female; *B*, male; *C*, male and female of natural size; *D*, vulva.

middle of which projects a spiculum 5 to 6 mm. in length. Specimens of the female have been noted of 100 cm. in length and 12 mm. in diameter.

They are both smooth, round worms, of a blood red color. The eggs are oval, measuring 40 by 64 micra. The shell is covered with small depressions, except at the two poles.

This parasite lives in the pelvis of the kidney. Some twelve authentic cases of human infection have been reported.

**Uncinaria duodenale** (*Anchylostoma duodenale*).—The male of this parasite measures from 8 to 10 mm. long and 0.4 to 0.5 mm. wide, and the female, 12 to 18 mm. long. The eggs are elliptical, with a smooth contour and transparent shell. They measure 55 to 65 micra in length and 32 to 45 micra in width. They are exceedingly abundant in the stools of persons carrying these worms and usually show evidences of segmentation. The eight cell stage is not usually passed unless the eggs have been exposed to the air, when they go on to develop into the worm in a short time if the fæces are kept warm and fluid.

The body of the parasite is cylindrical, tapering toward the anterior extremity. The mouth cavity is provided with two pairs of hook-shaped ventral teeth and one pair of dorsal teeth directed forward. In the bottom of the mouth there is a single dorsal conical tooth, which does not project prominently. A pair of long thin spiculæ extend from the sexual bursa of the male parasite. In the female the posterior end of the body is provided with a small pointed projection. The vulva is situated somewhat in front of the posterior third of the body.

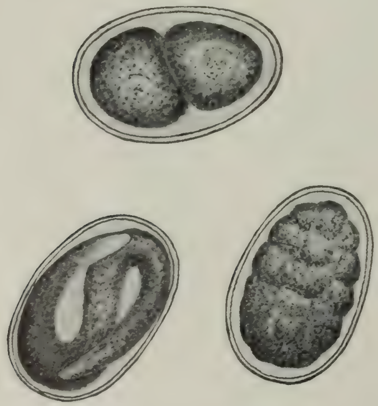

FIG. 111.—EGGS OF UNCINARIA AMERICANA IN VARIOUS STAGES OF DEVELOPMENT.

**Uncinaria Americana.**—Stiles[1] has shown that the specimens of Uncinaria obtained from the United States and the West Indies, show morphological differences which warrant their separation into a distinct species. The differences are as follow:

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[1] American Medicine, vol. iii, 1902, p. 777.

The ventral recurved hook-like teeth are absent from the mouth, their places being taken by a pair of semilunar plates. The dorsal conical tooth projects prominently into the buccal cavity. In the male the dorsal ray of the caudal bursa divides to its base, each branch being again split at its tip. In the female, the vulva is in the anterior half of the body, but near the equator. The eggs are slightly larger than the European form, measuring 64 to 72 micra by 36 to 40. In some cases they are partially segmented, in others they may contain a fully developed embryo, while still in the intestinal tract.[1]

Neither species of worm requires an intermediate host for the development of the parasite. The eggs mature in moist earth and the worms live for a long time in water. The infection of the human host takes place either by the drinking of contaminated water, or by the penetration of the skin by the embryos, as has been demonstrated by Looss.

The European form of parasite is abundantly found in Egypt, in Switzerland, where it infected the laborers on the Saint Gothard tunnel, in the lower Rhine district, where it infects the brick-makers, and in the mining districts of England and Wales. In Italy infection by this worm is very widely spread. The European variety has been observed in the Philippines.[2]

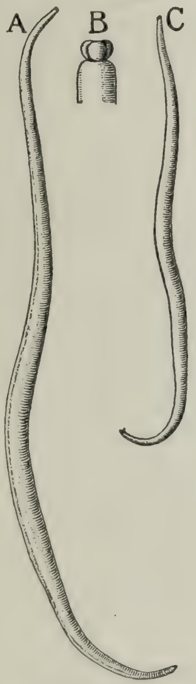

Fig. 112.—Ascaris Lumbricoides. *A*, female; *B*, head; *C*, male.

In America cases have been reported from a number of the Southern States, and occasionally from the miners in the anthracite coal district of Pennsylvania, while a large proportion of the popuiation of the West India Islands is infected by the American species of the worm. Occasionally, however, in the United States, the European worm is found, having been imported in the intestinal tract of the immigrant suffering from the infection.

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[1] An excellent article on the subject of uncinariasis in the United States, by *Smith*, may be found in the American Jour. Med. Sciences, vol. cxxvi, 1903, p. 768.

[2] *Craig:* American Jour. of Med. Sciences, vol. cxxvi, 1903, p. 798.

The diagnosis of the infection can easily be made by examining the stools for eggs, which are usually present in enormous numbers. The importance of the study of the parasite is that these worms may produce a fatal anæmia, due possibly both to the blood removed from the mucous membrane of the intestine and to the poison excreted by the body of the worm. If a diagnosis, however, is made in time, suitable remedies will remove the parasite and very frequently cure the patient.

**Ascaris lumbricoides.**—The male worm measures from 15 to 25 cm. in length and about 3 mm. in diameter. The posterior end is conical and curved ventrally in the form of a hook. The spiculum is 2 mm. long, curved, and somewhat spread out on its free end, while the cloacal opening is surrounded by seventy to seventy-five papillæ. The testicular tube is highly convoluted and can be seen through the transparent envelope of the parasite. The female is 20 to 40 cm. long and about 5 mm. thick. The posterior end is conical and straight. The vulva is at the junction of the anterior and middle third of the body. The paired uteri pass directly back to the posterior end of the worm. The convoluted ovaries are about 250 cm. long. The worms when freshly passed are a dirty yellow or brown color.

The eggs are elliptical, with a thick transparent shell usually covered with a granular albuminous coat. They measure 50 to 70 micra in length and 40 to 50 in breadth. They are deposited before segmentation takes place. The albuminous shell is frequently colored yellow by the pigment of the fæces. This shell is easily dragged off the egg by a slight pressure of the cover glass. The egg when passed in the fæces is still immature and shows no embryo, but only the yellow, granular yolk. The eggs which still retain their gelatinous envelopes are capable of resisting the action of the gastric juice and passing on to the intestine unharmed, where they may develop into mature worms; but the eggs which have lost their envelopes are destroyed by digestion.

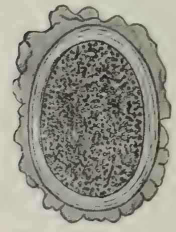

FIG. 113.—EGG OF ASCARIS LUMBRICOIDES.

The usual method of infection is for the eggs to develop in water until the embryo reaches a certain period of development, and then be taken into the body with the drinking water or the food. The worms inhabit the small intestine, but frequently crawl out of the anus or into the stomach, from which they may be vomited. Occasionally these round worms may cause a fatal obstruction to the glottis by crawling out of the œsophagus while the patient is asleep and entering the larynx. The worms may also cause jaundice by obstructing the common duct. They are frequently found in people of all ages.

**Oxyuris vermicularis.**—The threadworm is one of the most common of the parasites of the nematode group.

The male is about 4 mm. long, with a blunt tail, while the female measures 10 mm. and the tail tapers to a point. The worms inhabit the ileum, the caput coli, and the colon, while the mature females frequently wander out of the anus at night. They may also be found in the vagina, bladder, and nasal cavities.

The eggs are oval and measure 20 to 50 micra, and the embryos can often be distinguished in them. The eggs are passed in the fæces and transferred to the mouth by the food or the fingers. When they reach the stomach, the chitinous envelope is dissolved by the gastric juice and the embryo is set free to develop in the intestines. No intermediate host is required. Fourteen days after swallowing the eggs the worms may be found in a mature condition in the fæces. The primary infection is through the food or direct contact with an infected person. The worms are seen chiefly in young children, though they may be found in old persons.

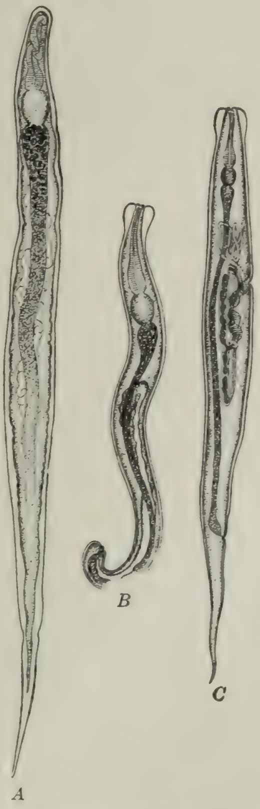

Fig. 114.—Oxyuris Vermicularis. *A*, female; *B*, immature female; *C*, male.

## VII. ARACHNOIDEA

**Sarcoptes scabiei**.—This minute parasite is the inciting agent of the itch. It is usually found in those portions of the body where the skin is thin, especially between the fingers and in the folds of the elbow and knee-joint. The tunnels made by the insect may vary from a few millimeters to several centimeters in length, and at the closed end the female parasite may be found. The tunnel contains eggs and small fæcal masses. The male is very seldom found.

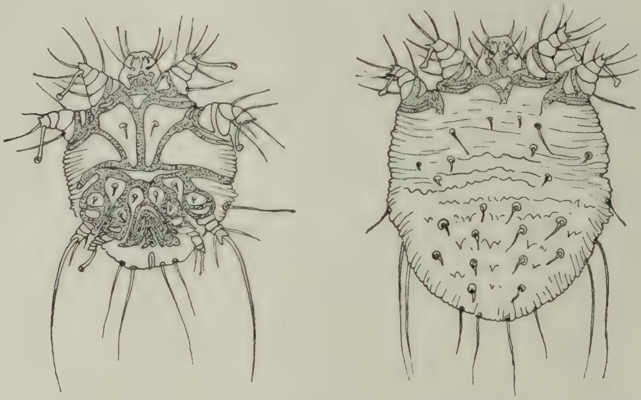

Fig. 115.—Male and Female of Sarcoptes Scabiei. (Braun.)

Infection may occur through contamination from clothing, but it is usually by direct contact. In order to demonstrate the parasite the tunnel should be opened with a needle and the female squeezed out on a slide, covered with a cover glass, and examined (Fig. 115).

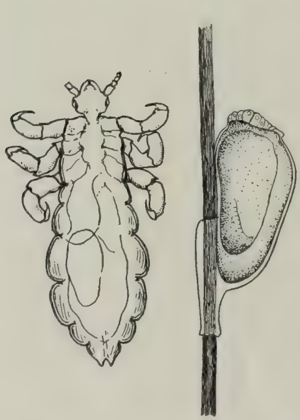

Fig. 116.—Pediculus Capitis and Egg.

A number of varieties of sarcoptes exist which may become parasites on man, but usually inhabit the domestic animals. They vary slightly in their size and form from the human itch insect.

## VIII. INSECTA

**Pediculus capitis** (*Head louse*).—The male is 1 to 1.5 mm. long, the female 1.8 to 2 mm. The color is a light gray or black. The eggs are about 0.6 of a mm. in length and are attached by the female to the hair, where they form the small whitish oval masses known as nits. (Fig. 116.) The pediculus capitis is usually confined to the hair of the head, but occasionally is found in other portions of the body.

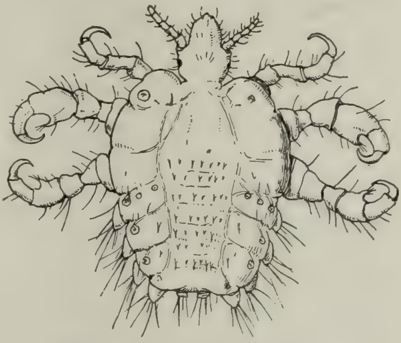

FIG. 117.—PEDICULUS PUBIS.

**Pediculus vestimenti** (*Body louse*).—The parasite measures from 2 to 4 mm. in length and is pale gray in color. The abdomen is wider than the thorax. The antennæ are longer than those in the head louse. The eggs are 0.7 to 0.9 mm. in length and are often deposited on clothes.

**Pediculus pubis** (*Crab louse*).—The male measures from 0.8 to 1 mm. long, the female about 1.1 mm. The flattened body is of a grayish color. The eggs are about 0.8 to 0.9 of a mm. in length and 0.4 to 0.5 of a mm. in breadth. They are deposited on the hairs of the pubis. This parasite is very rarely found on the scalp.

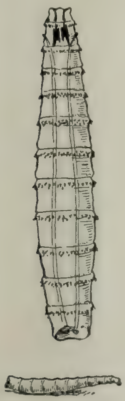

FIG. 118.—LARVA OF HOUSE FLY (Musca vomitoria).

**Intestinal Myiasis.**—The ova of the common house fly are not infrequently found in specimens of fæces, either as a result of contamination after passing or from the eating of imperfectly cooked food which has been infected by the ova of the fly. The phenomenon is unimportant from a clinical point of view, as no symptoms are caused, the specimens being chiefly derived from neurasthenic patients who have examined their own stools.

## IX. PARASITIC DISEASES OF THE SKIN

The following parasitic diseases of the skin are due to the hyphomycetic or fungus group, and occasionally microscopical examination offers points of diagnostic value.

**Favus.**—The disease is due to the invasion of the epidermis of the hair follicles, usually of the scalp, by the mycelium and spores of the fungus Achorion Schönleinii. The spores gain access to the deeper layers of the skin by means of the hair follicles and develop around the hair shaft, forming a cup-shaped mass of a sulphur yellow color. The hair bulb and root sheaths are infiltrated with the fungus, which may grow to considerable length without septa or break up into comparatively small fragments. The mycelium branches at right angles. The mature spores measure 3 to 10 micra in diameter.

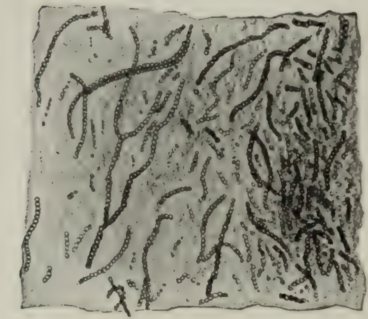

FIG. 119.—T. MEGALOSPORON ECTOTHRIX. (Crocker.)

The best way to demonstrate the parasite is to soften one of the fragments of the crust with a few drops of 10 per cent. caustic potash or soda. The preparation is covered and examined with a high-power lens. It is not always possible to make a positive diagnosis of favus by the microscope, as the fungus may resemble to a certain extent that of ringworm, so that recourse to cultures may be necessary.

**Tinea tonsurans** (*Ringworm of the scalp*).—The fungus of this disease may be divided into two main groups, Trichophyton microsporon and Trichophyton megalosporon.

**Trichophyton microsporon** (*Microsporon Audouini*).—This parasite occurs throughout the hair as a long jointed mycelium, from which branches pass out into the surface, where ectospores are

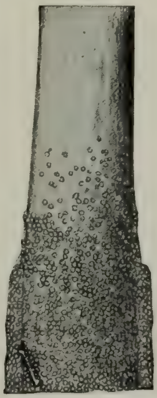

FIG. 120.—MICROSPORON AUDOUINI, SHOWING LAYER OF ECTOSPORES.

formed. The surface of the hair may be covered by a closely packed mass of ectospores from 2 to 3 micra in diameter, which are not as a rule arranged in a straight line.

**Trichophyton megalosporon endothrix.**—This parasite usually invades the hair of the scalp. The mycelium in the endothrix occupies the center of the hair shaft, commencing at the root and running throughout the whole length. It branches from time to time and seldom breaks through to the outer surface of the hair. Spores are abundantly formed and lie ~~in~~ long lines.

**Trichophyton megalosporon ectothrix.**—This parasite usually involves the hairs of the beard, inducing the lesion known as tinea sycosis. It reaches the human subject directly or indirectly from the horse, cat, dog, or other domestic animals. The fungus is usually limited to the intrafollicular region of the hair and forms a sheath between the hair and the follicle. Nearly all of the mass may be found attached to the hair when it is drawn out. The spore is in chains, but is less regularly formed than in the endothrix. It measures 3 to 8 micra in breadth and 4 to 12 micra in length.

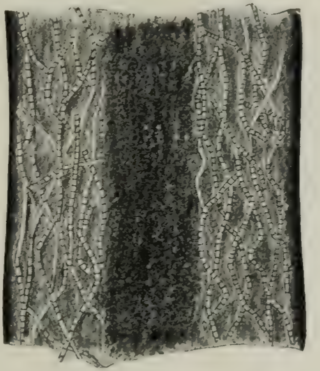

Fig. 121.—Trichophyton Megalosporon Endothrix.

**Tinea circinata** (*Ringworm of the body*).—This lesion may be caused either by the micro- or megalosporon.

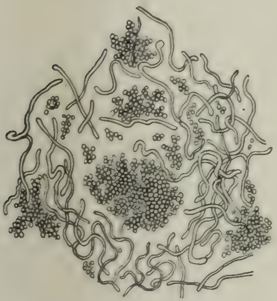

Fig. 122.—Microsporon Furfur.

**Tinea versicolor.**—The fungus which produces this disease is known as the Microsporon furfur. The diagnosis can be made by scraping off a small amount of fungus from the yellowish-brown patches and examining in a dilute solution of caustic potash. The mycelia are not usually branched or jointed, but lie in a close feltwork. When fully developed they show the conidia at their extremities. The conidia are as a rule larger than those of the ringworm, being just a trifle under the diameter of a red blood corpuscle and have a large nucleus. They are usually collected in rounded or grape-like masses. The parasite does not enter the deeper portions of the skin, but remains confined to the cornified layers of the epithelium.

**Oïdium albicans.**—This organism is the inciting agent of a condition known as thrush or soor. It is not infrequently seen on the mucous membrane of the buccal cavity and tongue of marantic children. The fungus forms a whitish or yellowish membrane which can be pulled off the mucous surface without much difficulty. When examined microscopically the membrane is found to consist of epithelial cells, leucocytes, and detritus lying in the meshes of a branching fungus. The mycelial threads are segmented and show spore-like areas at each end. The conidia (spores) form irregular scattered masses throughout the meshwork. The fungus may penetrate between the epithelial layers of the mucous membrane of the mouth and give rise to considerable inflammation, as is shown by a red hyperæmic border about the area to which the parasite is attached. The fungus is also found on the mucous membrane of the nose, in the sputum, and attached to the esophagus and inner surface of the stomach, and also to the vaginal mucosa. Quite rarely it invades the body and gives rise to general infection causing localized abscesses in the body. For instance, Heubner[1] describes a case in a young child, the course of the disease resembling somewhat a diphtheritic infection. The tonsils were covered with a yellow, dry, membrane containing spores and mycelial threads. The tracheal lymph nodes were invaded by the fungus, as well as the kidneys and lungs.

**Oïdiomycosis cutis** (*Blastomycosis*).—The disease usually appears as a nodular eruption, which becomes pustular and breaks down into an ulcer. The discharge from the ulcer or scrapings from the surface, when examined fresh or stained with aqueous thionin, show double contoured organisms, often budding. They measure from 12 to 14 micra in diameter. Smaller forms, measuring about 4 micra, are also found. Chains containing four to six segments are occasionally observed.[2]

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[1] Deut. med. Woch., 1903, p. 581. See also *v. Hibler:* Cent. f. Bakt. Origin, Bd. xxxvi, 1904, p. 505.

[2] For further details see a very complete paper by *Ricketts:* Journal of Medical Research, N. S., vol. i, 1901, p. 374.

# PART V

# ORAL AND NASAL SECRETIONS

## I. SALIVA

THE saliva is a mixed secretion derived from a number of glands, the submaxillary, sublingual, and the mucous glands of the mouth. It may vary, therefore, in composition, depending upon the relative amounts of fluids of various physical and chemical qualities secreted by each gland.

The macroscopical examination of the saliva affords no information of clinical importance.

**Microscopical Examination of Saliva.**—The saliva contains a variable number of cells, chiefly those derived from the epithelium of the mouth. These cells are large and flat, with a small nucleus. Slightly smaller cells, with relatively larger nuclei and granular cell bodies, are known as salivary corpuscles. Red and white blood cells in small numbers may be occasionally seen. The salivary secretion also contains a large number of bacteria, yeasts and molds.

The most important of the first group, from a morphological point of view, is the Bacillus maximus buccalis, or Leptothrix buccalis, which is arranged in long strands and is characterized by turning blue when treated with iodine solution. An actively motile spiral organism, the Spirochæte buccalis, is especially abundant in the tartar from the teeth and the coating of the tongue. Another less frequently seen spirochæte is a very small organism, the Spirochæte dentium (see Fig. 62).

A large number of other bacteria are also present in the saliva, the most important of which are the Staphylococcus pyogenes aureus and the pneumococcus. The Streptococcus pyogenes is also occasionally met with, and the diphtheria bacillus may be found in persons who have been in contact with cases of diphtheria, but who have not the disease. The smegma and timothy bacillus may also be found in the mouth.

In children the thrush fungus, oidium albicans, is not infrequently met with, forming white patches on the inner surface of the mouth and on the pharynx and tonsils. For a further consideration of this and other pathogenic bacteria, see section on Exudates.

Amœbæ are occasionally found in the saliva. They are most often derived from the material contained in carious teeth [1] or in the pus from osteomyelitis of the inferior maxilla. They measure 6 to 32 micra, and do not resemble the Entamœba coli, but rather E. histolytica. (See p. 339.)

**Chemical Examination of the Saliva.**—The reaction is usually considered faintly alkaline, from the blue color produced by the action of the saliva on litmus paper, but it is frequently neutral or even faintly acid. It is not alkaline to phenolphthalein. Under pathological conditions the saliva may have a marked acid reaction, especially in connection with febrile and digestive disturbances and diabetes.

**The specific gravity** of the saliva varies from 1.002 to 1.006.

**The amount** is not accurately known, but is assumed to be from 1 to 2 liters in twenty-four hours.

The saliva contains sodium and potassium chloride, calcium carbonate, traces of phosphates, and very small amounts of potassium sulphocyanide and nitrite. Mucus, traces of albumin, and a diastase, ptyalin, are also present.

The presence of sulphocyanide can be demonstrated by adding to the saliva to be tested a small quantity of hydrochloric acid and then a very dilute solution of ferric chloride, and warming gently if necessary. An orange or reddish color shows the presence of the sulphocyanide. If the mixture is shaken up with ether, the color is transferred to the ether and the saliva is decolorized.

For the identification of the nitrites we may use a thin starch paste containing potassium iodide and faintly acidulated with sulphuric acid. If the nitrites are present in the saliva the iodine will be set free when the former is mixed with the reagent, and the starch will assume a blue color.

The ptyalin may be determined by allowing the saliva to act on a small amount of thin starch paste. After a few seconds the presence of a considerable quantity of sugar can be made out in the fluid by the use of Fehling's solution. This sugar is maltose, the chief product of digestion by ptyalin. A trace of dextrin is also formed.

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[1] *Prowazek:* Entamœba buccalis, Arbeiten a. d. Kais. Gesundheitsamte, 1904, Bd. xxi, p. 42. *Flexner:* Johns Hopkins Hosp. Bull., 1892, vol iii, p. 104.

In splitting up the starch a series of substances are produced, the first being amylodextrin, which takes a blue color when treated with iodine. The next stage is erythrodextrin, which assumes a violet or mahogany-brown color when mixed with Gram's solution. The third is achroödextrin, which is not colored with iodine solution. The final stage is maltose. The process takes place in fluid of faintly alkaline, neutral, or faintly acid reaction.

## II. NASAL SECRETION

The amount of nasal secretion normally produced is very slight, being just sufficient to moisten the surface of the mucous membranes of the nasal passages. An increase in the secretion is produced by chemical irritation, or by chilling of the body surface, or by bacterial infections.

In order to obtain suitable specimens of nasal secretion it is better not to use that which may be obtained by blowing the nose on a cloth. A speculum should be inserted in the anterior nares and small quantities of the material removed by means of a sterile platinum loop or a swab of sterile cotton.

In acute coryza the alkaline secretion contains a large number of flat epithelial cells, and also cylindrical, ciliated epithelium, leucocytes, and bacteria. When chronic, a large amount of mucus is also present, containing large numbers of pus cells.

**The pathogenic organisms** which occur in the nose are chiefly the tubercle, lepra, and glanders bacilli. The diphtheria bacillus is also frequently found in the nose, inciting a membranous inflammation, which may also extend to the pharynx. The meningococcus intracellularis has also been found. A capsulated diplococcus has been described as quite constantly present in ozæna.[1] Ascarides and flagellata have rarely been seen. Charcot-Leyden crystals are occasionally found in the nasal secretion after an attack of bronchial asthma, and in chronic inflammation of the mucous membrane with formation of polypoid growths.

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[1] *Abel:* Zeit. f. Hygiene, 1895, Bd. xxvii, p. 89.

**Nasal concretions** are usually composed largely of vegetable fibers introduced by inhalation, cemented by inspissated mucus, and hardened by deposition of lime salts. They rarely reach a large size.

**Cerebrospinal fluid** occasionally is discharged through the nose in cases of fracture of the base of the skull. Its recognition is a matter of some difficulty, but if a fluid is obtained which contains either no albumin or only traces, and also a reducing substance, it is probably of cerebrospinal origin.

# PART VI

# THE SPUTUM

## I. GENERAL CONSIDERATIONS

By sputum is understood the secretion derived from the bronchi and alveoli of the lung, and not any other secretion from the nasopharynx. Inasmuch as the exudate from the bronchial mucosa differs greatly from that derived from the alveolar surface, so the sputum from the lung tissue and from the bronchi varies in its consistence and other physical properties.

As a preliminary to the examination of the sputum, care should be taken in the avoidance of error by first instructing the patient to cleanse the mouth, in order not to contaminate the sputum with particles of food; and, secondly, in order to obviate the presence of nasal and salivary secretions, to see that the patient furnishes for examination only the fluid which comes on deep coughing, and to avoid carefully having any quantity of saliva in the mouth at the time the sputum is brought up. Care should be taken also to have the bottle, or, what is better, the small paper cups for the collection of sputum, perfectly clean, so that no admixture of foreign substances may render the diagnosis more difficult.

Oral, nasal, and pharyngeal secretions can usually be recognized by their large content of mucus and by the presence of large, flat, pavement epithelial cells from the mucous membrane of the mouth or pharynx.

## II. MACROSCOPICAL EXAMINATION

**The Amount.**—The quantity of sputum expectorated in twenty-four hours varies greatly. It may be only a few cubic centimeters or may reach as high as 1,000 c.c., or even more. Very large quantities are seen in cases of œdema and abscess or gangrene of the lungs, and in acute bronchitis with bronchiectatic cavities. Cases of phthisis with large cavities often produce large quantities of sputum, while after the perforation of an empyema into the respiratory tract, it is not uncommon to see one, or even two, liters of pus brought up from the pleural cavity through the bronchi. Occasionally also large quantities are seen in a purely bronchial affection, as the so-called bronchorrhœa, or after removing large quantities of fluid from the pleural cavity.

**The Consistence.**—The consistence of the sputum varies greatly. In bronchial asthma, in acute catarrhal conditions, and also in pneumonia, the sputum is very thick and tenacious, and the cup holding it can often be inverted without spilling any of the contents. On the other hand, in œdema of the lungs the sputum is quite fluid and resembles blood serum. In abscesses of the lungs, in gangrene, or in abundant sputum from a large cavity in cases of phthisis, the sputum may be quite fluid and separate on standing into an upper layer of clear serum and a lower layer of pus.

**The Color.**—The sputum is either perfectly colorless and transparent, or a pale yellow or greenish color in uncomplicated cases of bronchitis. In pulmonary œdema it is quite transparent, with a faint pink shade from the dissolved hæmoglobin. In bronchial asthma and in pneumonia, the sputum may be like the white of an egg. In other cases the sputum is more opaque, due usually to the large number of leucocytes.

A red color is due to the presence of blood. It is usually bright red when the blood is derived from a rupture of a small vessel, and the brightness of the color is dependent largely upon the time the sputum has remained in the lung. In pulmonary gangrene a dirty brownish red is often seen, due to the presence of methæmoglobin or hæmatin. Dark brown sputum may be colored by bilirubin or may be due to blood, as in the prune-juice sputum of severe lobar pneumonia.

Bright green sputa are noticed where bile pigment has been mixed with the sputum, due to the perforation of a liver abscess into the lung. Green sputa may also be noticed in connection with a pneumonia, in which resolution has taken place slowly and the blood pigment has been altered into hæmatoidin, or, what is presumably the same thing, bilirubin. The bilirubin is then changed by oxidation into biliverdin.

Blackish sputum may be noticed in coal handlers or persons living in a smoky atmosphere. The sputum which is derived from the perforation of an amœbic liver abscess into the lung shows a color resembling anchovy sauce.

**The Odor.**—Most sputa are practically odorless. Under certain conditions, however, the odor may be horribly offensive, especially in cases of gangrene of the lung or large bronchiectatic cavities.

Sputa may be classified in general as serous, mucous, mucopurulent, and bloody. As a rule, a pure sputum of a single variety is rare, most specimens containing varying amounts of mucus, and pus, and often traces of blood.

For the proper macroscopic examination of the sputum it is best to pour the specimen out in a shallow dish and place the dish on a black background. A large-sized Petri dish, as used by bacteriologists, is very convenient.

It is important to recognize the small cheesy particles which are brought up in the sputum of a case of phthisis, for these small masses contain large numbers of bacilli when the rest of the sputum may be fairly free from them. It is often difficult, however, to distinguish these cheesy masses from small, partially digested food particles, so that in cases of doubt it will save time if the mass is touched with a drop of Gram's solution, which will turn the particle of bread a blue color, bread forming the most deceptive of these small masses.

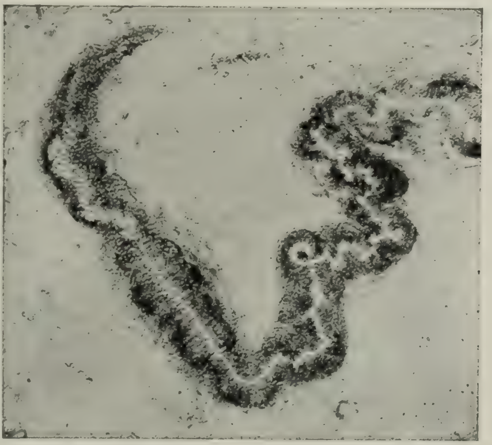

Fig. 123.—Curschmann's Spiral, showing Central Thread.

**Fibrinous Casts.**—Complete casts of the bronchi are occasionally seen in fibrinous bronchitis, in pneumonia during the process of consolidation of the lung, and in diphtheria. They are often composed of a large number of branches and may be 5 to 15 cm. long. (See page 404 and Fig. 127.)

**Curschmann's Spirals.**—These are long, clear threads, which are just visible to the naked eye, and under the microscope can be seen as mucous threads of a spiral form, usually wound about a clear central strand. Many eosinophile cells and Charcot-Leyden crystals may be present in the mucus. The crystals may be found when the spiral is coughed up, or they may form only after the specimen has been kept for twenty-four hours or more. The spirals are most frequently found in bronchial asthma.[1]

Small **fragments** of **lung tissue** and **cartilaginous rings** from the smaller bronchi are seen occasionally in gangrene of the lung. They are usually dark in color, and on microscopical examination show no structure except the elastic tissue network of the lung substance or the clear transparent substance of the cartilage.

**Echinococcus Cysts.**—Fragments of the walls of echinococcus cysts or their contents have been coughed up in the sputum on rare occasions. The presence of the scolices and the hooks furnishes an absolutely pathognomonic evidence of the nature of the cyst as does the laminated membrane (see Figs. 101 and 102). Some of the nematode worms or their ova are occasionally found in the sputum.

**Concretions** and **foreign bodies** are occasionally coughed up in the sputum.[2] The former are usually derived from the calcareous deposits seen in old tuberculous nodules either in the lung or in the bronchial lymph nodes.

## III. MICROSCOPICAL EXAMINATION

**Leucocytes,** chiefly of the neutrophile variety, are found in every sputum, but in bronchial asthma the eosinophile forms are likely to be abundant, and occasionally basophile cells may be present. Free eosinophile granules may be found in large numbers. The leucocytes may contain particles of carbon, fat, or hæmatoidin, showing that they act as phagocytes.

**Red blood corpuscles** are to be found in almost every sputum, especially in persons having a chronic pharyngitis from any cause and who cough violently in bringing up the sputum. Small quantities of blood may be derived from the trachea or the bronchial mucous membrane in the process of coughing, and are without clinical significance. Larger amounts of blood, such as would be visible to the naked eye, may be derived from the same sources, but it is always well to consider them as due to pulmonary tuberculosis until physical and other examinations negative this supposition. A great many persons suffering with early tuberculosis are allowed to drift on without careful examination of the sputum or the chest until the disease has so advanced that climatic treatment is useless, simply because the physician assumes that small amounts of blood in a bronchitic sputum are due to a chronic smoker's pharyngitis.

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[1] *Curschmann:* Deut. Arch. f. klin. Med., 1883, Bd. xxxii, p. 1; also Bd. xxxiv, p. 578.

[2] *Atlee:* Bronchial Concretions, Amer. Jour. Med. Sci., 1901, vol. cxxii, p. 49.

The blood corpuscles may be present in the sputum in a well-preserved form, or the hæmoglobin may be dissolved out and altered into the various decomposition products of that substance, hæmatoidin being evident if the sputum be of a yellow or a bright green color. Large amounts of blood are easily recognized and are indicative of advanced changes in the lung, such as gangrene, abscess, phthisis, heart disease, pneumonia, or œdema. Traces of blood may be demonstrated in the sputum by the tests of Donogány and Weber (see pages 329 and 570).

**Epithelial Cells.**—These cells are often present in the sputum and are of various types. Tall cylindrical cells, especially if ciliated, are derived from the larynx, trachea or bronchi. Large flat cells are derived from the alveolar epithelium and frequently show fatty degeneration or what is known as myelin degeneration. The latter is evidenced by the cells containing large amounts of a clear, highly refractile substance, which may show fine concentric striations. The cell may be invisible and only the myelin appear as a large, irregular mass. The large flat epithelial cells also contain, especially in chronic heart disease, a pigment derived from the blood which is known as hæmosiderin. This pigment gives an iron reaction when treated with a dilute solution of potassium ferrocyanide and hydrochloric acid. At times this iron reaction is not given and the substance appears to be hæmatoidin.

**Elastic Tissue.**—Elastic fibers are found in the sputum when there is rapid destruction of the lung tissue. This breaking down of the lung occurs most commonly in pulmonary tuberculosis, but the elastic fibers are also found in abscess of the lung and in gangrene. In some cases of gangrene only a few or no fibers may be found, and it is supposed that the ferments, which are present in the gangrenous tissues, digest the elastic tissue. The detection of this elastic tissue is a matter of considerable diagnostic value, especially in the early stages of pulmonary tuberculosis. If abundant, it can easily be demonstrated in the sputum by pressing a small amount of the latter substance out between two glass plates and examining with a low power for the fibers. They show an alveolar structure exactly as they are arranged about the alveoli in the lung substance. The fibers are especially abundant in the small cheesy particles described on page 390, but if one of these can not be found, a small portion of the thicker, purulent part of the sputum should be examined.

If the fibers can not be found in this way, a portion of the thick part of the sputum should be boiled with about an equal amount of 10 per cent. NaOH until the mixture becomes soft and fluid. The softened mixture is then allowed to stand for twenty-four hours and the sediment examined microscopically. The elastic fibers have a high power of resistance to the action of strong acids or alkalies, and while the other constituents of the sputum are completely destroyed by the alkali, the elastic fibers remain and can be easily picked out from the débris in the sediment.

The fibers may be stained by orcein [1] after washing the sediment with a little distilled water to get rid of an excess of alkali. A few c.c. of the dye are mixed with the deposit, adding a few drops of hydrochloric acid if the color turns violet. The tube is then warmed in boiling water for five minutes and the contents decolorized by acid alcohol. (Strong HCl, 5 c.c.; 80 per cent. alcohol, 1,000 c.c.) Care must be taken not to have elastic fibers introduced into the sputum from the food, as these fibers have exactly the same characteristics, except that they are not arranged in the alveolar form, since lung tissue does not ordinarily occur in food.

A convenient method when the fibers are fairly abundant is to stain a smear of sputum with Weigert's elastic tissue stain.[2] A very thick smear of the suspected sputum is made on a slide and allowed to dry in the air. The slide is then placed in a Coplin jar filled with the dye, the alcohol of which fixes the specimen. The slide should remain in the dye for about half an hour. It is then removed and decolorized for a few seconds in 3 per cent. hydrochloric acid in alcohol until the color is almost entirely removed. The slide is then dried and a thin layer of cedar oil smeared over it with a brush in order to render the preparation transparent. When this result is obtained the slide may be examined with a medium power lens for the elastic fibers. They will be stained a deep purple, in contrast to all débris and vegetable fibers, which are not stained.

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[1] See Appendix.

[2] See Appendix.

The older writers found elastic tissue in some 90 per cent. of their cases, but at present the disease is usually in an early stage when the diagnosis is made, and the writer has been able to find elastic tissue in but a small per cent. of fairly active cases, unless large quantities of sputum were treated with alkali. The Weigert staining method only occasionally gives a positive result.

**Fibrin.**—This substance may be occasionally seen in the sputum, especially in lobar pneumonia. It forms a delicate network or small casts of the alveoli in fresh preparations. If further proof of its nature is wanted, the specimen may be fixed on the slide by alcohol and stained with Weigert's stain for fibrin. The stain and the technique will be found in the paragraph on Actinomycosis (page 403).

### CRYSTALS IN THE SPUTUM

**Fatty Acid Crystals.**—These are the most abundant crystals in the sputum of gangrene and chronic phthisis. They form long, slender, pointed rods, usually collected in bundles. They are soluble in ether and melt on gently warming the slide.

**Cholesterin.**—This substance is seen in sputum from chronic lung abscesses, empyema, and rarely in chronic phthisis with cavities. The crystals may be readily identified by their rhomboidal form and notched angles. A little strong sulphuric acid and Gram's solution run under the edge of the cover will color the crystals red and blue.

**Charcot-Leyden crystals** are seen chiefly in the sputum of bronchial asthma, though occasionally met with in the sputum from cases of acute bronchitis. They are often seen embedded in the Curschmann's spirals. Morphologically they are very similar to, though not identical with, the crystals seen in the spermatic fluid. They form pointed, colorless octahedra, which are soluble in warm water and acetic acid, but not in formalin, and take up eosin, thionin, and other stains. They do not give a strong double refraction when examined by polarized light as do the spermatic crystals.

**Leucin and tyrosin** are formed in pus by the decomposition of the proteids, and may be expected, therefore, in the sputum from an empyema which has perforated into the lung or from a liver abscess which is draining in the same manner. More rarely these crystals are seen in other conditions in which pus appears in considerable quantities in the sputum, notably in abscess of the lung.

The crystals are usually seen only when the purulent fluid is allowed to evaporate upon the slide. Leucin is insoluble in ether and is thus distinguishable from fat globules. Other tests are given in the chapter on the Urine (page 457).

**Hæmatoidin crystals** are met with only rarely in the sputum, and then only when blood has been extravasated into the lung alveoli and remained there for some time. The crystals are rhomboidal in form and often have small curved filaments projecting from the angles. Amorphous masses of hæmatoidin are also seen, which can be recognized by the easy solubility of this substance in chloroform. The color of the crystals is a bright yellow, approaching a brown in large masses.

**Calcium oxalate** and **triple phosphate crystals** are occasionally met with. They may be identified by their morphology and reactions as given under Urine (page 607).

## BACTERIA IN SPUTUM

The best stain to use in order to obtain a general idea of the morphology of the sputum is the Jenner stain. The specimen should be spread in a very thin layer on a slide, allowed to dry in the air, then stained with the Jenner solution for three minutes. It should be washed thoroughly in distilled water for fifteen to twenty seconds until the excess of methylene blue is removed. All bacteria, with the exception of the acid-resisting groups, take the stain. The granules of the neutrophile and eosinophile leucocytes are well demonstrated, and the pigmented cells from the lung alveoli show well-stained nuclei. Charcot-Leyden crystals take up the eosin component of the stain.

Another method which has been found useful[1] is a modification of the Gram procedure, using the following solutions:

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[1] *Smith:* A Method of Staining Sputum for Bacteriological Examination, Boston Med. and Surg. Jour., vol. cxlvii, 1902, p. 659.

1. Aniline oil-gentian violet;
2. Gram's iodine solution;
3. Saturated aqueous solution of eosin;
4. Loeffler's alkaline methylene blue;
5. Alcohol-ether, containing 95 per cent. alcohol, four parts, ether six parts.

The stain gives good results only when the sputum is perfectly fresh and has not been treated with any preservative agent.

A very thin smear is made and the specimen fixed with heat. It is then flooded with aniline oil-gentian violet and warmed until steam is given off. The excess of the dye is washed off with Gram's solution. The slide is covered with the same and again steamed. The excess of the dye is then removed with 95 per cent. alcohol. The preparation is then washed for a few seconds in the alcohol-ether mixture.

After washing in water the smear is stained for a few seconds in the aqueous eosin solution, which is washed off with the Loeffler's methylene blue. This stain is also warmed to the steaming point. The preparation is again washed in 95 per cent. alcohol, then in absolute alcohol, which is followed by xylol. The preparation is mounted in dammar and examined with an oil immersion lens.

The appearance of a specimen stained by this method is as follows: The protoplasm of the polynuclear leucocytes, lymphocytes, and other cells takes the eosin stain. The nuclei stain with Loeffler's blue. The Gram staining organisms are black or violet, while those bacteria which are negative to Gram take the Loeffler's blue. The capsules are stained by eosin.

The unmodified Gram stain is occasionally useful in identifying bacteria by their morphological appearances, and the Weigert fibrin stain may also be used.

Special stains are given under the bacteria which they are used to demonstrate.

### THE TUBERCLE BACILLUS

This is the most important pathogenic germ in the sputum, and the ease with which it can be detected has rendered an early diagnosis of the disease with which it stands in causal relation a matter within the ability of every practitioner. The methods of staining the tubercle bacillus in a specific manner are dependent upon a property of the organism by which it takes up aniline dyes with great difficulty, and when thoroughly stained resists the action of decolorization with equal stubbornness. At first this reaction was considered specific for the tubercle bacillus, but recently other groups of bacteria have been found to have the same peculiarities, notably the smegma and timothy groups.

It is of considerable importance, especially in early cases where the quantity of sputum is small, to instruct the patient as to the care which is necessary in obtaining uncontaminated specimens. The most suitable material is that coughed up on rising in the morning, and great care should be taken to previously cleanse the mouth and teeth and to avoid contaminating the specimen by nasal or pharyngeal mucus or by saliva. If large numbers of flat epithelial cells are found in the preparation and tubercle bacilli can not be found immediately, it is best to reject the specimen and obtain one not containing contaminations.

The selection of the portion of the sputum to be examined is of importance, as it will often enable the experienced observer to find tubercle bacilli where the beginner fails. As a rule, the thick purulent portions of the sputum are to be selected and spread on a slide or cover glass with a stout platinum needle or a platinum spatula, made by hammering out a thick platinum wire. Care should be taken that the smear is extremely thin, as the thicker portions decolorize imperfectly and may lead to false results. If the sputum is poured into a Petri dish and placed on a piece of black paper, one may occasionally note, especially in the rapidly advancing cases, small cheesy masses derived from the expectoration of portions of a softened lung, or fragments from the walls of cavities, from which an almost pure culture of the bacilli may often be obtained. Some experience, however, is necessary to recognize these fragments, and the beginner is apt to select particles of food or the expectorated plugs from the tonsillar lacunæ.

**Ziehl-Neelsen Stain.**—The smears may be made either upon slides or cover glasses, the slides being more convenient where many specimens are to be examined in succession. The covers are fixed by passing three times through a flame, the slides by passing nine times. The specimens may be stained with carbol-fuchsin[1] by gently heating the slide, which has been covered with the dye, or, what is preferable, by placing the slide face downward in a Petri dish containing the warmed staining fluid. For the cover-glass preparations small porcelain staining dishes are convenient, and a few c.c. of the carbol-fuchsin may be heated in one of these dishes until the steam begins to rise, and then the fixed cover-glass is laid face down on the surface of the dye, where it will usually float. The warming should be continued for about three minutes. During this time the tubercle bacilli will have been colored and also all of the bacteria in the preparation. In order to differentiate the tubercle bacilli it is necessary to remove the color from the other germs. This may be accomplished by washing the slide or cover glass in 5 per cent. nitric, 5 per cent. sulphuric, or 1 per cent. hydrochloric acid in 85 per cent. alcohol. The preparation is treated with acid until, after washing with water, the smear appears of a bright pink color. The surplus dye is then extracted with 90 per cent. alcohol, until no more color can be seen to dissolve in the alcohol, and the smear assumes a grayish tone, with only a slight pink shade. The preparation is then washed with water and stained for a few seconds in 1 per cent. aqueous methylene blue, in order to color the leucocytes and bacteria which may be present. The slide is washed in water, dried with blotting-paper, and a drop of immersion oil added, and the search for the tubercle bacilli begun, preferably with a mechanical stage. It is not necessary to cover the preparation or to mount in balsam. A 1–12 oil immersion furnishes the most convenient magnification, though one who is familiar with the morphology of the bacilli may often obtain satisfactory results when using a high aperture 3 mm. dry lens and a strong eyepiece.

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[1] See Appendix.

**Gabbett's Method.**—For rapid work the decolorization and the counter staining can be carried out in one operation as recommended by Gabbett. The fluid is prepared by dissolving 2 grams of methylene blue in 100 c.c. of 25 per cent. sulphuric acid. The stained slide is then covered with this mixture for one minute. At the end of this time the preparation is washed and the red color due to the carbol-fuchsin will have entirely disappeared, and in its place there will be a diffuse blue from the methylene blue of the decolorizing fluid; while if the preparation be examined microscopically the tubercle bacilli will stand out from the background as bright red rods. The red color may still be retained in the thick portion of the smear, in which the decolorizing agent has not had time to penetrate. These thick portions are, therefore, unfit for microscopical examination. Gabbett's method, while it is convenient for rapid work in municipal laboratories, where large numbers of specimens have to be examined in a comparatively short time, is not so satisfactory as that given above, for the reason that strong acid, combined with methylene blue, is very likely to decolorize a certain number of the tubercle bacilli, so that if these are few in number a serious error may result.

**Pappenheim's Method.**[1]—This procedure may be used as a routine and offers the advantages of excluding from consideration the smegma bacillus, which is occasionally found in the sputum in cases of gangrene of the lung. It does not, however, decolorize certain acid-resisting bacilli present in tap water or in distilled water which has been kept for some time.

The sputum is spread on a slide and fixed by passing nine times through a flame. As soon as cooled the slide is stained with hot carbol-fuchsin for two minutes. The surplus dye is then poured off without washing the specimen and the decolorizing solution is poured over the slide, allowed to drain off slowly, and the process repeated five times.

The slide is then washed off in water, dried with blotting-paper and in the air, and examined with an oil immersion. Tubercle bacilli are stained red; smegma bacilli blue.

The decolorizing solution is made up by dissolving 1 gram of corallin (rosolic acid) in 100 c.c. of absolute alcohol and then saturating the mixture with methylene blue. Twenty parts of glycerin are finally added.

The appearance of the tubercle bacillus, when stained by either of the methods given above, is that of a long, slender rod, frequently curved, which appears to lie on the surface of the smear and not so deep as the other morphological elements stained with the methylene blue. They measure in general from three to four micra in length (about half the diameter of a red cell), though longer forms up to eleven micra are not uncommon. Short thick bacilli are also seen, especially in acute processes. In the bodies of the bacilli, from chronic cases of tuberculosis, there are frequently found irregular oval, highly refractile areas, which have in the past been considered to be spores. Some observers[2] still consider these to be resistant spore-like bodies, which may remain alive and give rise to infections in tissues in which the tubercle bacilli themselves can not be demonstrated by staining. The irregular staining of the bacilli seems to be more marked in those preparations which have been decolorized by the use of strong acids. Occasionally the appearance is so marked that the beginner may be led to consider the bacillus as composed of a chain of coccus-like members. They are especially abundant in the contents of old tuberculous cavities, in which also one may occasionally find branching forms of bacilli.

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[1] Berl. klin. Woch., 1898, p. 809.

[2] *Czaplewski:* Lehrbuch der klin. Untersuchungsmethoden, 1904, p. 384.

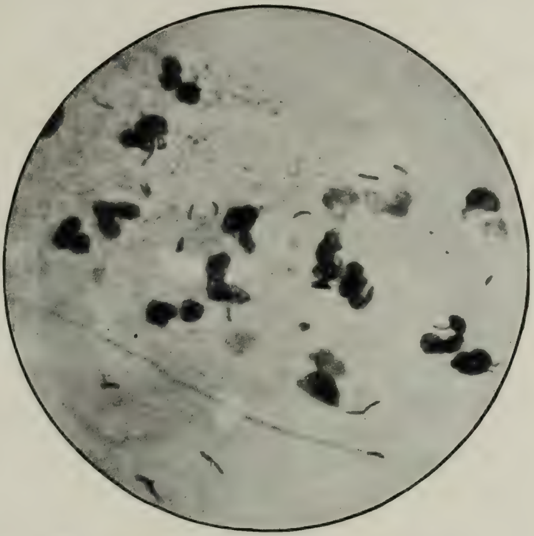

Fig. 124.—Tubercle Bacilli from Sputum.

The number of the bacilli present in the preparation offers no clew to the stage of the disease nor to the rapidity with which the case is progressing. Old cases with cavities may show only a few bacilli, or none at all may be found; while an early case with scarcely any signs in the lungs may give a sputum which contains enormous numbers of bacilli. It is therefore unimportant to attempt to determine the number of bacilli which are present in the sputum, and misleading to base prognostic statements on such numbers when obtained.

If bacilli can not be found in the sputum of a suspicious case, after examining a number of slides, it is well either to inject a guinea-pig subcutaneously with some of the material, or to attempt to obtain bacteria from a considerable quantity of sputum by softening the fluid and then concentrating the bacilli.

**Biedert's Method.**[1]—About 10 c.c. of sputum are diluted in a beaker with 90 c.c. of water. The mixture is then heated over a flame and 10 per cent. sodium hydrate added in small quantities, stirring in the mixture until the stringy masses of mucus have dissolved completely. The amount of sodium hydrate added should be as small as possible, only sufficient to soften the mucus, in order that the tubercle bacilli present may not be altered in their staining qualities.

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[1] Berl. klin. Woch., 1886, p. 713; also, Deut. med. Ztg., 1891, p. 337.

After softening is completed, the fluid is allowed to stand for a moment, a few drops of 1 per cent. phenolphthalein are added, and the mixture is accurately neutralized with dilute acetic acid. The whole is then poured into twice its bulk of strong alcohol, allowed to stand, and then centrifugalized. It may be necessary to use some egg albumin or some of the *patient's own untreated sputum* to fix the bacilli to the slide.

**Antiformin Method.**[1]—Equal volumes of sputum and 15 per cent. antiformin solution are mixed in a small bottle, and vigorously shaken. In a short time the sputum will be softened and all bacteria except the tubercle bacillus, dissolved, with the exception also of certain acid-resisting organisms present in tap water and in distilled water which has been kept for a long time. The ordinary acid-fast bacilli, such as the smegma bacillus, are dissolved. The softened mixture can be centrifugalized directly and the sediment washed with physiological salt solution, and again centrifugalized, or it can be poured into two volumes of strong alcohol and centrifugalized. Smears are made in the regular way, fixed on a slide by heat, and stained. The washing is necessary in order to remove the excess of strong alkali which interferes with the fixation of the bacilli on the slide and with their staining qualities. This method is much more satisfactory than the Biedert given above, and will probably entirely replace other concentration procedures.

Another method which has proved satisfactory is as follows:[2] Five, ten, or twenty c.c. of sputum are mixed in a test tube with an equal volume of 50 per cent. antiformin. The mixture is boiled for a moment, while being shaken constantly. The sputum softens almost immediately. Ten c.c. of the softened sputum are placed in a centrifuge tube, and 1.5 c.c. of a mixture of 12 volumes of chloroform and 9 volumes of absolute alcohol are added. The tube is corked, and the mixture is shaken and centrifugalized. The bacilli are found in a layer on the surface of the chloroform.

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[1] *Uhlenhuth:* Med. Klinik, 1909, v, p. 1296; *Kawai:* Med. Klinik, 1911, vii, p. 142.

[2] *Loeffler:* Deutsch. med. Wchnschr., 1910, xxxvi, p. 1987.

A full review of the different procedures has been published by Beitzke.[1]

### LEPRA BACILLUS

In many cases of leprosy it is possible to demonstrate the specific bacillus in the sputum or in the nasal secretion. Sticker [2] found lepra bacilli in the nasal secretion of 127 out of 153 cases of leprosy examined. In his opinion the mucous membrane of the cartilaginous portion of the septum of the nose is the site of the primary lesion of leprosy. Kolle [3] was able to verify the frequency of the nasal infection in leprosy, but he is somewhat more guarded in his views concerning the site of the primary lesions.

In order to demonstrate the bacilli it is merely necessary to make a thin smear of the nasal pharyngeal secretion, dry, fix, and stain with carbol-fuchsin. The smears should be decolorized for a short time with acid, decolorization completed with alcohol, and the specimen counterstained with methylene blue. The bacilli may be single or lie in large masses.

The writer has been able to demonstrate lepra bacilli in several cases which he has had the opportunity to study, but noted in one person, on whom the diagnosis of leprosy had been made by another physician, that the acid-resisting bacteria found were not so easily decolorized as the lepra bacilli usually are by the application of acid and alcohol. Further investigation showed that these bacilli were smegma bacilli, which were present in the nasal secretion and had led originally to an erroneous diagnosis.

Tubercle bacilli must be excluded by animal inoculation in doubtful cases, though usually the easier decolorization of the lepra bacilli, and the fact that they take up aqueous solutions of fuchsin or gentian violet in a short time at room temperatures, will allow of a differentiation.

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[1] Hygienische Rundschau, 1902, p. 1.

[2] Münch. med. Woch., 1897, p. 1063.

[3] Deut. med. Woch., 1899, p. 647.

### SMEGMA BACILLUS

The presence of acid-resisting bacilli in the sputum has been noted by a number of observers, and Fraenkel[1] and Pappenheim[2] have called attention to their presence in cases of gangrene of the lung.

Bacilli of the same group have been found in the crypts of the tonsils, in suppurative discharges from the ears of children, and, according to Moeller[3] and Lichtenstein,[4] they may occasionally be found in simple non-tuberculous bronchitis. The smegma bacilli found under these circumstances resist the action of 16 per cent. sulphuric acid for thirty minutes, and in some cases, of strong alcohol for eight hours. The smegma bacillus is not pathogenic to animals, so that the inoculation of a guinea-pig with the sputum will furnish definite information. Frequently, however, it is of importance to determine the nature of the infection more promptly than it can be done by animal inoculation, so that special differential stains must be used. The writer has always had satisfactory results with Pappenheim's method, as given in the previous paragraph.

**Bunge and Trantenroth**[5] recommend the following stain for differentiating tubercle from smegma bacilli:

After fixation of the smear, the fat is removed by soaking the slide in absolute alcohol. Then the preparation is laid in a 5 per cent. solution of chromic acid for fifteen minutes. The chromic acid is then carefully washed out in several changes of water. The slides are colored with carbol-fuchsin, decolorized with 16 per cent. sulphuric acid for three minutes, and then counterstained for five minutes in a concentrated alcoholic solution of methylene blue. According to these authors the tubercle bacilli retain the color, and the smegma bacillus is always decolorized.

In carrying out any of these special stains, however, it is always wise to make a second smear of known tubercle bacilli on the same slide as that containing the suspected material. This smear is then submitted to the same procedures as the one containing the bacillus to be tested, and by examining the slide one can determine whether the decolorization of the tubercle bacilli has been carried too far. A similar smear of smegma bacilli is often of use in determining the exact point of decolorization of this group of bacteria.

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[1] Berl. klin. Woch., 1898, pp. 246 and 880.

[2] Berl. klin. Woch., 1898, p. 809.

[3] Deut. med. Woch., 1898, p. 376.

[4] Zeit. f. Tuber., Bd. iii, 1902, p. 197.

[5] Fort. d. Med., Bd. xiv, 1896, p. 889.

It must be remembered, however, that different smegma bacilli vary greatly as to the time during which they will resist the action of acids and alkalies. Some are decolorized almost immediately; others withstand the action for a long time.[1]

### TIMOTHY BACILLUS

The timothy bacillus is another bacillus which has the power of resisting the decolorizing action of both alcohol and acid, and also produces a lesion in guinea-pigs, which resembles that of true tuberculosis. If, however, a second animal be injected with material from the first, no further development of pseudo-tubercles will result. The cultural characteristics of the germ easily distinguish it from the other members of this group, for it grows abundantly on ordinary media. It is present in the mouth, and is derived from butter and milk, in both of which numbers of these bacilli are commonly found. The writer knows of no morphological method which will certainly differentiate these bacteria from tubercle bacilli. The question is of no great moment, because they are not found in sputum from the lungs. Usually the bacilli are a good deal larger than the tubercle bacillus and frequently show branching forms.

### ANTHRAX BACILLUS

The sputum of cases of pulmonary anthrax may occasionally contain considerable numbers of anthrax bacilli, the identification of which may partially rest upon morphological criteria. The specimens should be spread, fixed, and stained either with Loeffler's methylene blue or by the Gram method. In the Loeffler stain the peculiar concave or square ends of the thick bacilli are well defined. Each bacillus measures from five to ten micra in length and from one to one and a half micra in breadth. They may be single or arranged in chains of three or four members.

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[1] *Coles:* Jour. of State Med., 1904, p. 216, states that smegma bacilli resists Pappenheim's decolorizer for four hours, tubercle bacilli for twenty-four hours, other acid resistant bacilli only for two hours.

The bacillus retains the gentian violet of the Gram stain, though old and swollen individuals may be partially or completely decolorized. To render the diagnosis more complete, the cultural identification of the bacillus should be carried out and also inoculation of suitable animals with recovery of the specific bacillus from the blood of the animal used. For this purpose white mice are especially convenient, as after subcutaneous inoculation of anthrax material at the root of the tail, bacilli can be demonstrated in the blood in large numbers in twenty-four to forty-eight hours. The inoculation incision should not be too deep, as confusion may result from the death of the animal from the bacillus of malignant œdema, which somewhat resembles the anthrax bacillus, but is distinguished by its strictly anaerobic growth.

### MICROCOCCUS CATARRHALIS

Cocci of this species are occasionally seen in the sputum in acute pulmonary conditions which resemble severe influenza. Smears from the sputum show the pus cells to be filled with small diplococci, which can not be distinguished from the gonococcus by stains or morphology, as they are negative to Gram. Cultural characteristics distinguish these cocci from the meningococcus and the gonococcus, but the methods are too complicated for the practitioner.[1]

### STREPTOCOCCI

Organisms of the streptococcus group may be found in the sputum from cases of acute bronchitis, acute lobular and bronchopneumonia, and in the sputum from patients with advanced pulmonary tuberculosis. In the latter condition they are in all probability responsible for the so-called hectic fever and the rapid advance of the process in the lungs. They can be identified provisionally by their morphology and by the fact that they are positive to Gram.

### STAPHYLOCOCCI

Staphylococci of various species are present in the sputum of persons with chronic bronchitis and chronic pulmonary tuberculosis. A probable diagnosis can be made from the morphology and the positive Gram stain. Their identification is of no diagnostic importance.

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[1] For a full discussion of this subject see *Ghon* and *Pfeiffer:* Zeit. f. klin. Med., Bd. xliv, 1902, p. 262.

### MICROCOCCUS TETRAGENUS

This is a large, encapsulated coccus, which is found with great regularity in sputum from chronic pulmonary tuberculosis with cavities. It is arranged in packets of four individuals. It is positive to Gram. The exact relation of this coccus to the tuberculous process has not been made out. It is, as a rule, but slightly pathogenic to man.

### PNEUMOCOCCUS LANCEOLATUS

The pneumococcus lanceolatus is found in the mouth of healthy persons, in sputum of acute and chronic bronchitis, in tuberculous sputum, and, finally, in large numbers in the sputum of acute pneumonic conditions, chiefly those of a lobar type. It is evident that its identification in the sputum is of little diagnostic value unless it is present in pure culture.

The morphological appearance of the pneumococcus is that of two short, conical bacilli, with their bases in contact and the whole surrounded by a delicate capsule. They may occur in chains or more commonly in pairs. The germ is positive to Gram. The presence of the capsule is the most important means of differentiating them from strepto- and staphylococci in exudates, but unfortunately it is often difficult to obtain a capsule stain.

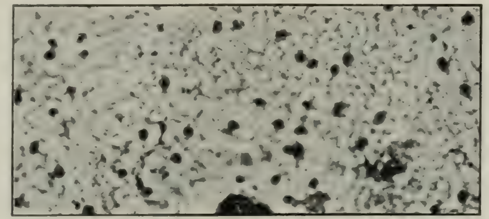

FIG. 125.—PNEUMOCOCCUS IN SPUTUM, SHOWING CAPSULES STAINED BY HISS' METHOD. Magnified 1,000 diameters.

In sputum and in pus, especially the pus from an empyema of long standing, large numbers of degenerated diplococci without capsules may be found. Occasionally free capsules or capsules surrounding shrunken cocci may be abundant.

**Welch's Method.**[1]—1. Smear the sputum without the addition of any water, dry, and fix by a moderate amount of heat.

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[1] Bull. Johns Hopkins Hosp., 1892, p. 125.

2. Flood the smear with glacial acetic acid and immediately pour off.

3. Wash off the acid with aniline water gentian violet.

4. Replace the stain with 2 per cent. salt solution, or examine directly in the stain by covering and pressing out the excess of dye, so that enough light will pass through to permit of examination. Do not wash with water.

The capsules can be demonstrated only in fresh sputum, and not constantly in that.

**Hiss' Method.**—The writer has obtained more constant results with the following:

1. Smear the sputum in a very thin layer without the addition of water, dry, and fix by heat.

2. Cover the preparation with a mixture containing 5 c.c. of a saturated alcoholic solution of gentian violet in 95 c.c. of distilled water. Heat till steam begins to rise.

3. Wash off the dye with a 20 per cent. solution of copper sulphate.

4. Dry and mount in dammar.

### BACILLUS MUCOSUS CAPSULATUS

Friedländer's bacillus is present in a certain small per cent. of cases of lobar pneumonia, of which it may be regarded as the inciting agent. The bacilli are encapsulated and act in a very variable way toward the Gram stain. In the sputum they are often positive, while in the first subculture made from the sputum they are generally negative.

### TYPHOID BACILLUS

In a number of cases developing pneumonia during the course of typhoid fever, the typhoid bacillus has been isolated from the sputum. Microscopically they appear as short, thick rods, which can not be distinguished from a large number of bacilli of similar morphology, which are negative to Gram. Culture methods only can furnish a positive diagnosis.

The sputum, which is usually hæmorrhagic in character, should be plated on Hiss' medium, and the thread-like colonies fished at the end of twenty-four hours and transferred to Hiss' tube medium. Bacteria from the tubes showing a characteristic clouding should then be tested with a typhoid serum of high agglutinating power. If the bacilli are promptly agglutinated, they are probably typhoid bacilli, and further determinations should be made of their cultural characteristics.

### PLAGUE BACILLUS

In the sputum of persons suffering from the pneumonic form of plague an enormous number of the characteristic bacilli may be found. The diagnostic points are that the bacilli are negative to Gram and that they take a marked polar stain. The best method of demonstrating the polar granules is to stain in a watery mixture of 2 per cent. methylene blue and 5 per cent. borax. This rather powerful stain should be allowed to act about half a minute, and the preparation is then decolorized by washing in alcohol.

The morphological diagnosis should always be confirmed by animal inoculations and culture methods.

### INFLUENZA BACILLUS

The morphology of the influenza bacillus is fairly characteristic to an experienced observer. The sputum shows large numbers of intra- and extracellular bacilli of very small size, often with polar granules. They decolorize by Gram and stain with difficulty in the ordinary aniline dyes. The best method is to use carbol-fuchsin, diluted 1 to 10, and allow the preparation to stain for ten minutes. Cultural methods are necessary for a definite diagnosis. They are occasionally found in the sputum of chronic bronchitis, long after the acute symptoms of the influenza have disappeared.

### GLANDERS BACILLUS

In the pulmonary form of glanders the bacillus may be found in the sputum, but except for the fact that they decolorize by Gram, they possess few morphological characteristics.

Cultures and animal inoculations are necessary for a final decision. The animal of choice is the guinea-pig, which should be inoculated in the groin with a moderate amount of the suspected material. One of the swollen lymph nodes of the inguinal chain should then be incised and a second male guinea-pig inoculated intraperitoneally. On the second or third day after inoculation, if the glanders bacillus is present, the animal will develop an acute orchitis. Death usually occurs from eight to fifteen days after the inoculation. Inasmuch as the orchitis is not absolutely characteristic of the glanders bacillus, cultures should be made from the infected material.

This method is somewhat longer than that by directly inoculating the material into the peritoneal cavity, but many animals die from peritonitis when the fresh material is placed in the abdominal cavity.

### FUNGI IN THE SPUTUM

Not infrequently long branching threads are met with in the sputum, some of which are due to fungi of the Aspergillus or Mucor group, and others to the Streptothrix group.[1] Unfortunately it is often difficult to decide between fungi accidentally introduced into the sputum before examination and those which are brought up from the lung. The morphological appearances are occasionally sufficiently characteristic to permit of a probable diagnosis, especially when the masses of fungus form greenish or brownish microscopic granules, with well-developed mycelial threads, conidia, and spores.

### ACTINOMYCES HOMINIS

This fungus, which frequently gives rise to a disease known as lumpy jaw in cattle, has occasionally been seen to infect the human subject with involvement of the lungs. The process is a necrotic one, and the muco-purulent sputum contains elastic tissue and small sulphur yellow granules which are visible to the naked eye. These granules are small masses of the ray fungus and should be picked out of the sputum and crushed under a cover glass. When examined under these conditions a central indistinctly granular mass is visible, from the periphery of which project a series of club-like rays.

Usually the morphology is sufficient for a diagnosis, but the sputum may be collected and hardened in formalin, embedded and sectioned, and the fungus stained with eosin and hæmatoxylin, or the isolated fragments of the actinomyces may be spread out on a slide, fixed by alcohol, and stained by Weigert's fibrin stain.

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[1] *Norris* and *Larkin:* Necrotic Broncho-pneumonia with Streptothrix, Jour. of Exp. Medicine, vol. v, 1900, p. 155.

For this purpose the smear is flooded with aniline water gentian violet solution for ten minutes. The stain is then poured off and the preparation blotted with filter paper to remove the excess of the dye. Gram's solution is then poured on the slide and allowed to remain for three minutes and the slide again blotted off. The decolorization is then completed by repeated treatment of the smear with a mixture of xylol one part and aniline oil two parts. The preparation can be examined as soon as it clears, or the excess of aniline oil may be blotted off and the preparation washed with pure xylol several times to remove all the aniline oil, then balsam and a cover glass applied. A light stain with eosin brings out the clubbed ends well. This is best carried out by using oil of origanum in which a little eosin has been dissolved for the last treatment, instead of the xylol.

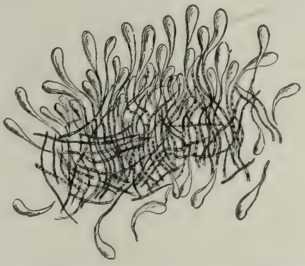

Fig. 126.—Actinomyces Hominis, Showing Club-shaped Extremities to the Rays. (Fresh preparation.)

## IV. THE SPUTA IN DISEASE

**Acute Bronchitis.**—The sputum is characterized by its mucous character, which is especially marked in the earlier parts of the disease, while later, as the inflammation tends to become chronic, the transparency is diminished and the color changes to a yellow or green, in place of the glassy mucous sputum of the early stages. At first only a few leucocytes and cells from the bronchi are to be seen, later numerous leucocytes and abundant bronchial epithelium, the latter sometimes ciliated, are to be observed. A small amount of blood is often present in all stages, but most abundantly at the beginning of the disease.

**Chronic Bronchitis.**—The sputum is in most cases much more abundant than in the acute forms and contains large numbers of polynuclear leucocytes, usually in a state of fatty degeneration or necrotic. The color of the sputum is a yellow green. Small numbers of alveolar epithelium and myelin masses may usually be found.

The quality and quantity of the sputum are altered if the formation of bronchiectatic cavities takes place. The sputum becomes more abundant and thinner, and on standing, separates into a layer of pus and one of mucus. If the cavities are large the sputum may undergo putrefaction, and large amounts of thin, foul-smelling, gray-green fluid may be coughed up. Enormous numbers of bacteria and degenerated leucocytes, fat needles, and occasionally leucin and tyrosin, may be found.

**The Sputum in Gangrene of the Lung.**—The sputum from cases of gangrene of the lung is characterized by its offensive odor, its color and fluidity. When poured into a conical glass such sputum will separate into layers, with a thick brownish deposit at the bottom, and a clear fluid in the middle, and a frothy layer on top.

Microscopical examination shows comparatively few cells and leucocytes, often numerous fatty acid crystals, and occasionally leucin and tyrosin. Elastic fibers and fragments of lung tissue are usually scanty. Autolytic ferments have been found in the sputum, which undoubtedly destroy the elastic fibers.

The number of bacteria present is often enormous, but except for the identification of the acid-resisting bacilli, which probably belong to the smegma or timothy group,[1] the morphological examination offers but few diagnostic points.

The Bacillus proteus vulgaris is frequently present, but must be identified by cultural means.

**Fibrinous Bronchitis.**—The characteristic symptom of the disease is the expulsion from the bronchi of more or less perfect casts of those tubules. These casts are composed of a mixture of mucus and fibrin. The formation of fibrinous casts may occur as an independent phenomenon in persons not suffering from any definite disease, and they may be brought up from time to time, especially after inhaling irritating dust.[2] Fibrinous casts of the bronchi are also seen in connection with acute or chronic pulmonary diseases; for example, influenza and tuberculosis. They have also been seen in uncomplicated valvular lesions and sclerosis of the coronary arteries, the specimen figured being from a case of this type.

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[1] *Rabinowitsch:* Zeit. f. Hygiene, Bd. xxvi, 1897, p. 90; also, Deut. med. Woch., 1900, p. 257.

[2] *Bettmann:* Amer. Jour. of Med. Sci., vol. cxxiii, 1902, p. 304; also, *Liebermeister:* Deut. Arch. f. klin. Med., Bd. lxxx, 1900, p. 551.

They may be coughed up in the course of an attack of spasmodic asthma[1] or during a thoracentesis.[2] They differ in structure from

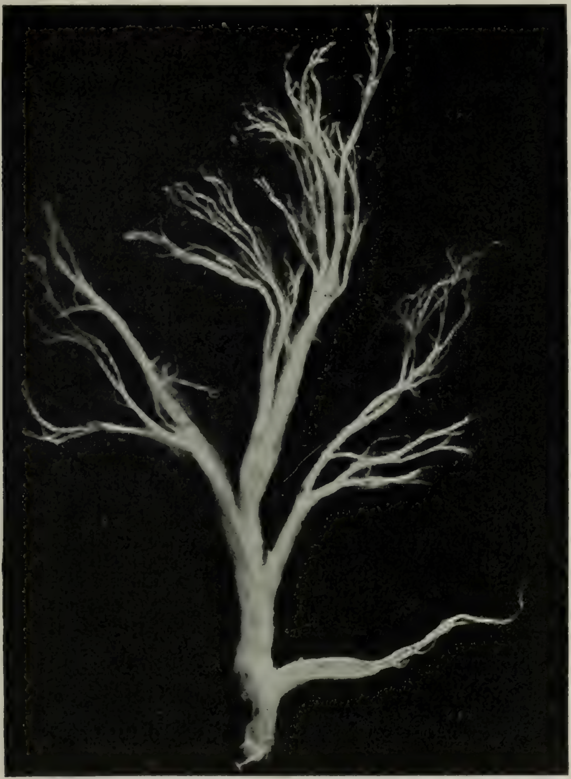

FIG. 127.—FIBRINOUS CAST OF THE BRONCHI. (Natural size.)

the soft masses of fibrin and necrotic cells which may be coughed up by persons suffering from tracheal diphtheria.

**Acute Lobar Pneumonia.**—In the early stages of the disease the sputum is of a yellowish-red color and very tenacious in its consistence. Microscopically it contains only a few red cells and degenerated leucocytes and possibly a pure culture or pneumococcus, often accompanied, however, by numerous streptococci.

In the second stage of the disease, when exudation is taking place in the alveoli, the sputum usually assumes a reddish color—

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[1] *Posselt:* Prag. med. Woch., 1899, p. 46.

[2] *Magenau:* Münch. med. Woch., 1902, p. 1697.

the so-called rusty sputum. It still retains its tenacious character, and the cup containing it can usually be inverted without losing any of its contents.

In the stage of resolution the sputum becomes thinner and more abundant and the color becomes yellow, like the sputum of bronchitis. The small fibrinous plugs of the second stage disappear, and the cellular elements of the sputum become more abundant and are in a condition of advanced fatty degeneration. Variations from these types are frequently met with, and sputum, containing large amounts of blood, are often seen, while in the sputum of grippe pneumonia the appearance is often that of a purely bronchitic exudate. In persons suffering from a chronic bronchitis and then developing pneumonia, the sputum retains to a great degree the characteristics of the bronchitic type and the rusty form of sputum may not appear.

If the patient suffers from jaundice during the course of a pneumonia, the sputum is likely to take on a bright green tinge from the oxidation of the bilirubin to biliverdin. This same form of sputum is observed in cases of delayed resolution when the exudate has been of an especially hæmorrhagic type. The blood pigment in this case is altered into hæmatoidin or bilirubin in the lung itself, and then the yellow pigment changed to the green form. The œdema of the lungs, which is so commonly seen in the latter stages of a fatal pneumonia, is characterized by a thin, serous, frothy fluid, which may be either of a faint yellow or pink or, what is more usual, of a dark brown, from the admixture of a considerable quantity of blood with the serous exudate. Microscopically only a few leucocytes and epithelial cells may be found.

**Empyema.**—The sputum which is derived from an empyema which has ruptured into the lung, is composed almost entirely of pus and is thin and liquid. It is usually brought up in large amounts. It may contain Charcot-Leyden crystals and leucin and tyrosin. Search should also be made in the sputum for the presence of the sulphur-yellow masses of the actinomyces.

**Abscess of the Liver.**—An abscess of the liver may perforate into one of the bronchi, in which case the sputum is apt to be of an ochre yellow or a red color, and contain abundant bilirubin needles and possibly the amœba dysenteriæ.

**Bronchial Asthma.**—The sputum from a case of bronchial asthma is very tenacious and is composed largely of mucus from the walls of the bronchi. This mucus may be in the form of Curschmann's spirals, and contain also large numbers of eosinophile cells and occasional Charcot-Leyden crystals. Basophile cells are occasionally seen in moderate numbers.

**Chronic Congestion.**—The sputum from chronic pulmonary congestion, such as occurs in uncompensated heart disease, usually contains numerous cells of varying form which are thickly filled with pigment of a brown color. This pigment often gives the iron reaction, but not always. The length of time it has remained in the lung seems to have something to do with the reaction, the older specimens not giving the test.

Similar cells are seen after a hæmorrhagic infarct, but the excessively bloody sputum, which accompanies this condition, differentiates it from the rusty brown sputum from a case of heart disease.

**Carcinoma or Sarcoma of the Lungs.**—The sputum from a case of malignant disease of the lungs may very rarely show fragments of the growth in sufficiently good preservation to allow of a diagnosis after sectioning and staining the masses of cells. Other definite microscopical or chemical evidences of a pulmonary tumor are not common. A point of some value is the presence in the sputum of masses of fat globules and fatty degenerated cells.

**Pulmonary Tuberculosis.**—The sputum in pulmonary tuberculosis is extremely variable both in quantity and structure, and also in the number of bacilli present.

In acute miliary tuberculosis the sputum is that of an acute or subacute bronchitis and contains no bacilli.

In early cases of phthisis there may be practically no sputum or only a small amount, which is coughed up on rising in the morning. It is usually clear and tenacious, with possibly a few yellowish areas where the leucocytes are more abundant. Occasionally such sputum is blood-streaked, or it may contain a large quantity of fluid blood, which has possibly been the first symptom of tuberculosis which the patient has noticed. Such sputa, in the writer's experience, occasionally contain enormous numbers of tubercle bacilli. No physical signs indicating that the lung is involved can be made out, and these patients often rapidly recover when transferred to a suitable environment.

When softening of the lung tissue or dilation of the bronchi is added to the purely miliary form, the sputum is that of an advanced chronic bronchitis, and contains in addition small fragments of broken-down lung tissue and elastic fibers. It is usually thick and purulent from the admixture of large numbers of leucocytes from the exudation.

In advanced cases after the development of cavities the sputum often contains oval or spherical masses of thick pus and mucus floating in a thinner, more serous fluid. This so-called nummular sputum is quite characteristic of the excretion of large cavities. The masses of pus contain enormous numbers of tubercle bacilli. Blood is seen in the sputum more often in the early cases than in the late, though in the late stages large hæmorrhages may occur from the erosion of a large vessel.

## PNEUMOCONIOSES

**Anthracosis.**—The sputum of the dwellers in large cities, especially where bituminous coal is burned in quantity, almost always contains a moderate amount of finely divided particles of carbon.

The expectoration of persons who smoke a great deal, especially those who inhale the smoke from the tobacco, is often of a grayish color or contains irregular blackish patches.

Typical anthracosis is seen chiefly in coal miners, where the lungs contain large deposits of coal dust, and the sputum may be almost black from this cause. Microscopic examination shows numerous free particles of coal, which can be recognized by their resistance to acids, alkalies, and reagents, and also many leucocytes and alveolar cells containing the black coal pigment.

**Siderosis.**—This condition is chiefly noted in workers in machine shops, especially where the air is filled with a large amount of iron oxide dust. The sputum is usually of a dark-brown color, and the presence of iron can be recognized by treatment with dilute solutions of hydrochloric acid, followed by potassium ferrocyanide.[1]

**Chalicosis.**—The sputum is usually that of a chronic catarrhal bronchitis, but it contains particles of stone, chalk dust or plaster of Paris, either free or inclosed in the cells.[2] The substances which incite chronic inflammation of the lungs of this type may be recognized by chemical reactions, especially the chalk dust and the sulphate of lime; but usually the clinical history will furnish all necessary data.

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[1] *Langguth:* Deut. Arch. f. klin. Med., Bd. lv, 1895, p. 255.

[2] *Betts:* Chalicosis pulmonum, Jour. of Amer. Med. Ass'n, vol. xxxiv, 1900, p. 70.

**Amylosis.**—Starch in the sputum is occasionally seen in bakers and may be recognized by its reaction with iodine. In those working in flour-mills the sputum frequently contains not only starch particles, but also small masses of vegetable cells derived from the covering of the wheat grain.

# PART VII

# THE URINE

## I. GENERAL CONSIDERATIONS

The examination of the urine is of great practical interest from a number of points of view, not alone for the diagnosis of kidney lesions but also for the recognition of changes in other organs.

The products, both organic and inorganic, of the destructive metabolism of the proteids are nearly all excreted in the urine, so that by an examination of this fluid we are in a position to determine the quantitative relationships between the intake and the output of nitrogen, as well as to detect the presence of substances which result from the imperfect digestion and absorption of the proteids or their putrefaction by the intestinal bacteria.

Under normal conditions the substances derived from the breaking down of the carbohydrates and fats of the food appear in the urine only in traces, but when the oxidizing power of the organism is diminished, carbohydrates are excreted in the urine, and also acetone and diacetic and $\beta$-oxybutyric acids, derived from the metabolism of the fats.

We may also study the substances which escape from the body under certain pathological conditions: such as, for instance, in diabetes, the excretion of sugar and the acetone group of compounds; in nephritis, the retention of urea and the chlorides; in cystinuria, not only the cystin but also the diamins; in myeloma, the Bence Jones' proteid.

We may also learn from the study of the urine many facts concerning the anatomical changes in the kidneys in disease. The composition of the urine may offer valuable suggestions as to the strength of the heart; the appearance in the urine of the bile pigments helps us in the diagnosis of diseases of the liver; increased intestinal putrefaction is made evident by the indoxyl which appears in the urine in large quantities; albumose may be present in internal suppuration.

The rate of absorption and excretion of drugs may often be determined by the examination of the urine, while poisons may occasionally be found in this fluid and thus lead to a diagnosis.

The study of the urine also affords us an opportunity to observe the methods which the organism employs to neutralize poisonous products, either introduced into the body from without or produced through a defect in the metabolism of the cells. For example, the conjugation of glycuronic or sulphuric acid with the various poisonous substances produced by the putrefaction of the proteids, such as indol, skatol, phenol, etc., is evidenced by an increased excretion of these conjugate compounds in the urine. The body thus protects itself from the poisonous action of these substances. As a good example of such protective power on the part of glycuronic acid, may be cited the observation of Hildebrandt,[1] in which it was noted that a fatal dose of a drug (thymotinpiperid) could be neutralized if the animal had previously received a suitable amount of dextrose. The urine under these conditions contained large quantities of the conjugate compound.

## II. PHYSICAL PROPERTIES OF THE URINE

### QUANTITY

The amount of the urine secreted during twenty-four hours is, on the average, between 1,000 and 1,500 c.c.; women passing about 200 c.c. less than men. Considerable fluctuations are seen, however, due to the amount of fluid taken in.

Less urine is passed during the warm months than during the cold, owing to the increased excretion of water by perspiration. The drinking of large quantities of fluid increases, while the taking of violent exercise diminishes the amount excreted. Large quantities of urine are passed by diabetic patients, in diabetes insipidus, during the resorption of large exudates, in the course of chronic diffuse nephritis, and as a result of strong psychical disturbances. A portion of the increase under these conditions is due to the fact that the irritability of the bladder is increased, and the frequency of micturition reflexly stimulates the kidneys to further increase in secretion.

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[1] Arch. f. exp. Path. u. Pharm., Bd. xliv, 1900, p. 278.

The amount is diminished in acute febrile diseases, in acute nephritis, in anæmia, atrophy of the liver and cardiac diseases, and after the loss of large quantities of fluid, either by hæmorrhage or in the course of an acute intestinal disease, such as cholera. Shock following the administration of an anæsthetic or operations on the genito-urinary tract frequently results in a great diminution in the quantity of the urine, or even entire suppression of the excretion, usually in connection with an unsuspected nephritis.

## COLOR

The color of the urine is normally of a pale yellow, and when examined by the spectroscope no absorption bands can be noted, only a general diminution in the intensity of the blue end of the spectrum. Pale urines are either very dilute or are seen in diabetes, with a high specific gravity, where pigment sufficient to color the fluid is not formed, or in chronic diffuse nephritis with greatly increased excretion. Dark-colored urines are usually concentrated or are febrile urines with an excess of urobilin. This substance can be detected by diluting the urine and examining it with a spectroscope, when the absorption band due to urobilin can usually be made out without difficulty. Red urines are colored either by blood or by the presence of pyramidon[1] or purgatin in a urine of alkaline reaction. Very dark-colored urines are noted after the absorption of carbolic acid, resorcin, or hydrochinon, or may be caused by the excretion of melanin derived from pigmented tumors of the body, and finally to the alteration of hæmoglobin into methæmoglobin. A greenish-yellow color may be caused by bile pigment or by urobilin, though the color due to the latter is usually more of a brownish tint. Occasionally it is impossible to distinguish the color due to the two substances and the differentiation must depend upon chemical tests. Yellowish urine is also passed after taking rhubarb, senna, or santonin, which is altered by the addition of an alkali to red. A pale greenish color is seen after the administration of methylene blue. Dark brown urine may contain methæmoglobin or hæmatoporphyrin or a mixture of these two substances. Milky urine is seen chiefly in connection with parasitic diseases such as filariasis or with the presence of Bilharzia.

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[1] *Apert:* Arch. gén. de Méd., 1904, p. 1665.

## TRANSPARENCY

The transparency of normal urine is quite characteristic. When the urine is passed directly after a heavy meal, a slight cloudiness is often visible, which can be cleared up by the addition of acid, although the reaction may be distinctly acid to litmus paper.

After several hours' standing a faint cloud appears in perfectly normal urine. This nubecula, as it is called, collects in the middle of the column of fluid and contains threads of mucus from the urinary passages, small granular cells, leucocytes, and surface epithelium from the bladder and urethra. Pathological urine is very frequently clouded by the presence of large numbers of bacteria or pus cells in cystitis, by epithelium and casts in nephritis, or by blood from hæmorrhage along the genito-urinary tract.

## ODOR

The odor of normal urine is aromatic and quite characteristic. The odors, which are of interest from a clinical point of view, are those of acetone in diabetes, of ammonia in cystitis, of indol and skatol in urine from a bladder which is connected with the rectum by a recto-vesical fistula. The odor of hydrogen sulphide may be noted in hydrothionuria and that of cacodyl after the administration of that drug.

## REACTION OF THE URINE

The reaction of the urine obtained from healthy persons on a mixed diet is slightly acid, except directly after a meal, when the sum of the bases present may be greater than that of the acids, and the urine may then turn red litmus paper blue. Phenolphthalein, however, is not reddened by such a urine, and it is probable that the bluing of the litmus is due to the separation of the $CO_2$ from the bases, with which it is in combination, by the action of the litmus in the paper. The true reaction in these cases is neutral.[1]

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[1] For a general discussion of this subject see *Friedenthal:* Zeit. f. Allg. Phys., Bd. i, 1901, p. 56, in which the writer claims that even the blood serum is not alkaline, but neutral or even faintly acid. Also *Auerbach* u. *Friedenthal:* Reaction des Harnes, Arch. f. Anat. u. Phys., 1903, p. 397; and *Folin:* Am. Jour. of Phys., 1903, p. 265.

The acidity is generally considered to be due chiefly to the presence of the dihydrogen phosphates, and in many specimens of urine an amphoteric reaction to litmus is present, due to the fact that the monosodium phosphate is acid in reaction and can exist in conjunction with the disodium phosphate which is alkaline in reaction. Red litmus paper is therefore turned blue, and blue litmus paper turned red by such urine.

The acids in the urine which contribute to the acidity are derived partly as oxidation products from the albuminous substances, partly from the oxidation of the phosphorus contained in the nucleins and lecithins of the food, and also from the volatile fatty acids, hippuric acid, the aromatic ethereal acids, and oxalic acid. Recent researches have also shown that uric acid may be present in a free condition in the urine and contribute slightly to the acid reaction. A vegetable diet may cause the urine to be alkaline to litmus paper, while an abundant meat diet produces a strongly acid reaction. The urine is often alkaline after frequent vomiting or washing out of the stomach, or hypersecretion of hydrochloric acid. Thirty-six to forty-eight hours after the crisis in pneumonia, a sharp diminution in the acidity of the urine is seen, which lasts one to two days, and corresponds to the period of increased elimination of sodium chloride. The change is due to an increase in the amount of the basic phosphates over the acid form.

Pathologically, acid urines may be seen after poisoning with one of the strong inorganic acids. Markedly alkaline urine may be due to the administration of large quantities of alkalies or of organic acids, or, finally, to the production of ammonia by the fermentation of the urine set up by bacteria present in the urine in cystitis. For the practitioner the mere determination of the presence of an excess of acid or alkali by means of litmus paper is sufficient, using preferably a neutral paper, which will be reddened by acids and blued by alkalies.

**Quantitative Determination of Acidity.**—The exact quantitative determination of the amount of acid or alkali present is a matter of some difficulty owing to the complicated relation between the acid and alkaline phosphates, but the simplest method is that of Naegeli[1] as modified by Folin[2], titrating with $^{N}/_{10}$ NaOH, using phenolphthalein and alizarin as indicators. The necessary reagents are:

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[1] Zeit. f. phys. Chemie, Bd. xxx, 1900, p. 313.

[2] Am. Jour. of Phys., 1903, p. 265. For a study of the quantitative relations between the "mineral" and "organic" acidity, the original paper should be consulted.

1-10 normal sodium hydrate,
1-10 normal hydrochloric acid,
0.5 per cent. solution of phenolphthalein in 50 per cent. alcohol,
1 per cent. aqueous solution of alizarin sodium sulphonate.

*a*. Twenty-five c.c. of the mixed twenty-four hours' urine, which has been prevented from fermenting by the addition of thymol, is mixed with 15 to 20 grams of potassium oxalate and shaken for about a minute. One or two drops of the phenolphthalein solution are added and the mixture is titrated with the decinormal sodium hydrate, until a faint pink tint remains permanent. The flask should be shaken during the operation. This is the point of neutralization, and the acid may be expressed in c.c. of decinormal solution or in grams of hydrochloric acid, remembering that 1 c.c. of a decinormal sodium hydrate solution is equivalent to 0.00365 grams of hydrochloric acid. By this method the average acid capacity of urine passed in 24 hours is found to be that due to 1.45 grams of hydrochloric acid, or 25 to 30 c.c. of $^{N}/_{10}$ NaOH for each hundred of urine.

*b*. By means of a second titration carried out with decinormal hydrochloric acid it is possible to determine how much acid must be added to the urine in order to cause a distinct acid reaction due to the presence of free acid, in which process it is necessary to transform the secondary phospates, urates and oxalates into acid salts. Carbonates are changed into chlorides; chlorides and sulphates remain unchanged.

Alizarin red is the most suitable indicator for the purpose of determining the acid capacity. Decinormal hydrochloric acid is added until the alizarin assumes a yellow color.

The number of c.c. of $^{N}/_{10}$ NaOH used in *a* gives the acid, that is, the amount of the phosphates, urates, and oxalates, which exist in the form of acid salts. The number of c.c. used in *b* gives the amount of phosphates, oxalates, urates, and carbonates present which exist as neutral salts, and therefore do not react to phenolphthalein. The sum of *a* and *b*, therefore, gives the total acid contained in the phosphates, urates, oxalates, and carbonates.

In order to obtain an expression for the amount of bases present, the results of *a* should be added to double the amount of *b*, which will give the total amount of alkali in conjunction with phosphates, oxalates, urates, and carbonates. The sulphates and chlorides are not included in this determination. If the urine has undergone alkaline fermentation the results will be incorrect, as phenolphthalein does not give a sharp end reaction under these conditions. Facts of diagnostic value are not obtained by the quantitative determination of the acidity or alkalinity of the urine.

## SPECIFIC GRAVITY

The specific gravity of the urine is an expression of the total solids in the urine, and is of use as furnishing a rough clinical estimate of this fact. Evidently the determination of the specific gravity of the urine from a few ounces of the urine taken without regard to meals, exercise, the amount of fluid recently consumed, or the total daily amount of urine passed, is of no value in making such an estimate. For the determination to be of any scientific value it must be made from a specimen obtained by collecting the whole urine for twenty-four hours, determining its bulk, and then obtaining the specific gravity from a portion of the mixed urine.

The specific gravity of normal urine lies between 1.012 and 1.024 when the quantity passed is about 1,200 c.c. In disease the specific gravity varies from 1.002 to 1.060. The low gravities are seen in diabetes insipidus, in hysteria, and in chronic interstitial nephritis, though the urine of a perfectly normal person may be quite as low for a few hours if large quantities of slightly diuretic fluids have been consumed. The high gravities, as a rule, are seen after prolonged operations, especially with ether as an anæsthetic, where there has been great loss of blood from hæmorrhage and of body fluids from perspiration. The very high figures are due usually to the increase of the urinary solids by the enormous quantities of sugar excreted by diabetics, which may rise to 100 grams to the liter of urine. A considerable quantity of sugar may, however, be present in urine of a specific gravity of not over 1.015 to 1.020, so that, while a high specific gravity should make one suspicious of sugar, a low one by no means excludes the possibility of its presence.

Approximate results may be obtained by the use of a urinary hydrometer, with a scale from 1.000, which is the specific gravity of water, to 1.060, the upper limit of the urinary density. These urinometers, as they are called, can be obtained with a small thermometer either in the bulb or fused into the side of the small cylinder in which the urine is placed. This adds greatly to the expense, but also greatly to the value of the results, which must always be corrected for the temperature at which the observation is made.

The urine is poured into the small cylinder which accompanies the urinometer and the foam removed from the top by means of a small strip of filter paper. The urinometer should be dry, and is to be lowered gently into the urine with a slight spin. The rotary motion will prevent any adhesion between the urinometer and the sides of the vessel, which might cause a serious error in the reading. This should never be made when the instrument is adherent to the vessel. The reading is to be taken from the lower portion of the meniscus, not from the highest point to which the fluid rises along the stem. The correction for temperature is roughly made, within the limits of error of the apparatus, by adding 0.001 for every 3° C. that the urine is above the point at which the urinometer is corrected, and subtracting the same amount for every three degrees the urine is below the point of correction. For example, if a specimen of urine have a specific gravity of 1.020 at 30° C., and the point of correction of the instrument be, as is usual, 15° C., it will be necessary to add 0.005 to the reading—i.e., the true reading is 1.025.

The following table taken from Bouchardat gives the necessary corrections for temperature, measured in degrees Centigrade, when using a specific gravity float corrected for 15° C.

| TEMPERATURE. | Normal Urine. | Glucose Urine. | TEMPERATURE. | Normal Urine. | Glucose Urine. |
|---|---|---|---|---|---|
| 5° C. | −0.9 | −1.3 | 21° C. | 0.9 | 1.2 |
| 6 | −0.8 | −1.2 | 22 | 1.1 | 1.4 |
| 7 | −0.8 | −1.1 | 23 | 1.3 | 1.6 |
| 8 | −0.7 | −1.0 | 24 | 1.5 | 1.9 |
| 9 | −0.6 | −0.9 | 25 | 1.7 | 2.2 |
| 10 | −0.5 | −0.8 | 26 | 2.0 | 2.5 |
| 11 | −0.4 | −0.7 | 27 | 2.3 | 2.8 |
| 12 | −0.3 | −0.6 | 28 | 2.5 | 3.1 |
| 13 | −0.2 | −0.4 | 29 | 2.7 | 3.4 |
| 14 | −0.1 | −0.2 | 30 | 3.0 | 3.7 |
| 15 | ... | ... | 31 | 3.3 | 4.0 |
| 16 | 0.1 | 0.2 | 32 | 3.6 | 4.3 |
| 17 | 0.2 | 0.4 | 33 | 3.9 | 4.7 |
| 18 | 0.3 | 0.6 | 34 | 4.2 | 5.1 |
| 19 | 0.5 | 0.8 | 35 | 4.6 | 5.5 |
| 20 | 0.7 | 1.0 | | | |

The decimal figures in the column are to be added to the fourth figure of the specific gravity as found by the urinometer. In other words, supposing the specific gravity of the urine to be 1.019 at a temperature of 30° C., it will be necessary to add three units to the 1.019; that is, the specific gravity at 15° C. will be 1.022. If the specific gravity, on the other hand, be 1.019 at 10°, it will be necessary to subtract five from the fourth decimal place in normal urine, and eight in glucose urine.

If the exact specific gravity of the urine is of importance, as it may be, for example, in testing specimens obtained by catheterization of the ureters, it is much better to use a pycnometer or a Westphal balance.

Another method is to use an hydrometer of special form, such as that designed by Saxe,[1] which requires only a few cubic centimeters of urine. (See Fig. 128.) The urine is placed in the reservoir in the lower portion of the hydrometer, the stopper is inserted, and the instrument is placed in distilled water. The specific gravity is read off on the scale.

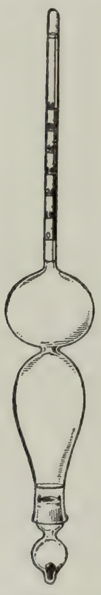

Fig. 128. — Urinometer for Small Quantities of Urine. (Saxe.)

**Pycnometer.**—In order to determine the specific gravity in very small quantities of urine, such as are obtained by catheterizing the ureters, or where an accurate determination is necessary, the pycnometer offers the most satisfactory results. The only drawback is that the use of this instrument requires a chemical balance sensitive to fractions of a milligram.

A number of types of pycnometer bottles are on the market, the most convenient of which is shown in Fig. 129.

The bottle is cleaned and thoroughly dried, then filled with distilled water. The thermometer is then inserted, which drives the excess of fluid out of the side neck. The fluid is then accurately adjusted to a mark engraved on the side neck, by means of a narrow strip of filter paper or a tapering pipette. The temperature of the fluid is read off and the bottle is weighed. The distilled water is then removed, the bottle cleansed and dried and weighed while empty. It is again filled with the urine to be tested, the temperature brought to the same point as that of the distilled water at the previous weighing, and the weight again determined. The weight of the urine is obtained by subtracting the weight of the empty flask, and its specific gravity by dividing the result by the weight of the water as previously obtained.

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[1] New York Med. Jour., 1903, vol. lxxviii, p. 739.

If the weighings are all made at the same temperature it is not necessary to repeat the weighing of the distilled water and the empty flask. These factors may be considered as constant, the only element to be determined being the weight of the urine, which should always be brought to a standard temperature. In this country it is convenient to assume a temperature of 18° to 20° C. as standard, though 15° C. is frequently used in Germany.

The results are accurate to the fifth decimal, while those obtained by the urinometer have an error of at least one point in the third decimal.

**The Westphal Balance.**—A much more convenient method of obtaining accurate determinations of the specific gravity of the urine and also of the specific gravity of the blood by Hammerschlag's method, and for the determination of the strength of solutions, is to use a Westphal balance.

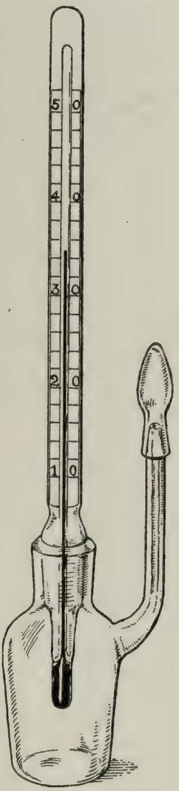

Fig. 129. — Pycnometer Bottle with Thermometer.

It consists of a support which can be levelled and also adjusted in height, and a balance with two arms of unequal length, one of which is divided into ten parts by a series of notches. The small Reimann float, which contains a thermometer to give the temperature of the fluid, is hung on the hook at the end of the graduated arm and the instrument adjusted by means of the screw at the foot until the two pointers on the short arm coincide. The weight of the thermometer, including the platinum wire suspending it, is 15 grams. Its bulk is so adjusted that it displaces 5 grams of distilled water at 15° C. To compensate the displacement due to pure water, a large counter-weight of 5 grams is hung on the hook just above the float. In distilled water at 15° C. the correctly adjusted instrument should again assume a balanced position. In urine it is necessary to add riders to the beam in order to produce equilibrium because of the greater density of the fluid displaced by the float. The smaller riders are so arranged that they weigh respectively 0.5, 0.05, and 0.005 grams. These are placed at various points on the beam until the balance is in equilibrium. When the two pointers coincide the position of the riders is read off.

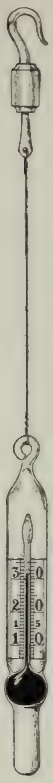

Fig. 130.—Reimann Float.

The rider weighing one-half a gram gives the second decimal, the one weighing fifty milligrams gives the third decimal, and the one weighing five milligrams gives the fourth when hung on notch 1. When the riders are placed on notches along the beam they indicate amounts one-tenth less than when hung on notch 1. If, for example, with a specimen of urine it is necessary to put on rider No. 1 at the notch 1, and rider No. 2 at the notch 5, the specific gravity of the urine is 1.015. If the rider No. 1 be placed at the notch 3, and rider No. 2 at the notch 9, the specific gravity is 1.039. Care should be taken to bring the urine to the proper temperature or correct by use of a table if the thermometer in the float does not indicate 15° C. The height of the instrument should be so adjusted that the loop of wire sustaining the float is just immersed in the fluid. Great care should be taken that the platinum wire and the thermometer are clean and free from grease. The platinum wire should be occasionally burned off in a Bunsen flame in order to remove impurities, and the float cleaned by soaking in a mixture of sulphuric acid and potassium bichromate.

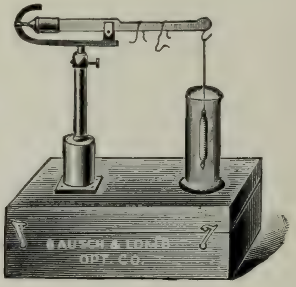

Fig. 131.—Westphal Balance. Simple form.

This form of balance is much cheaper than an analytical balance, and is convenient if a large number of accurate determinations are to be made for scientific purposes. For the clinician the urinometer is sufficient.

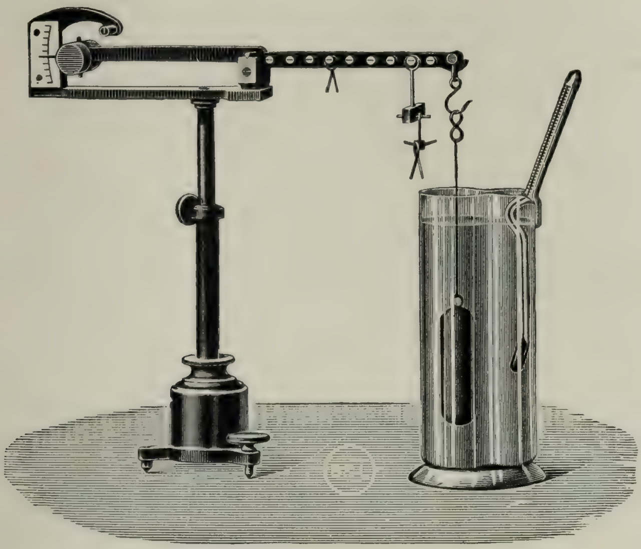

FIG. 132.—WESTPHAL BALANCE.

The total solids in the urine to the liter can be roughly estimated by doubling the last two figures of the specific gravity, or, more accurately, by multiplying by 2.2. For small children the factor is 1.6.

## III. CHEMISTRY OF THE URINE

### GENERAL COMPOSITION OF THE URINE

The composition of the urine varies greatly, but the urine of a healthy adult, about fifteen hundred c.c. in amount, will contain approximately sixty grams of solids, of which the inorganic constituents form about twenty-five grams, the organic about thirty-five grams.

1. The inorganic substances are:

| | |
|---|---|
| Hydrochloric acid (HCl), about | 9.35 grams |
| Sulphuric " ($H_2SO_4$) | 2.5 " |
| Phosphoric " ($P_2O_5$) | 2.5 " |
| Nitric " ($HNO_3$) | 1.0 " |
| Oxalic " ($C_2H_2O_4$) | 10 to 20 mg. |
| Sodium ($Na_2O$) | 7.9 grams |
| Potassium ($K_2O$) | 3.0 " |
| Ammonia ($NH_3$) | 0.7 " |
| Lime (CaO) | 0.3 " |
| Magnesia (MgO) | 0.5 " |
| Iron | 1 to 2 mg. |

2. The organic substances consist of

| | |
|---|---|
| Urea, about | 30.0 grams |
| Uric acid | 0.7 " |
| Creatinin | 1.0 " |
| Hippuric acid | 0.7 " |

Other organic substances amount to 2.3 grams, which is made up of small quantities of

Purin bodies.
Oxaluric acid.
Volatile fatty acids $\left\{\begin{array}{l}\text{acetic.}\\\text{formic.}\\\text{butyric.}\end{array}\right.$
Lactic acid.
Acetone.
Carbaminic acid.
Oxyproteic acid.
Alloxyproteic acid.
Succinic acid.
Traces of carbohydrates.
Traces of proteids.
Glycuronic acid.
Glycero-phosphoric acid.
Benzoic acid.
Hydrogen sulphocyanide.
Phenol- and p-cresol-sulphuric acid.
Pyrocatechin-sulphuric acid.
Indoxyl-sulphuric acid.
Indoxyl-glycuronic acid.
Indol-acetic acid.
p-oxyphenylacetic acid.
p-oxyphenyl-propionic acid.
Glyoxylic acid.
Allantoin.
Pigments.
Ferments.
Inosite.

3. Abnormal pathological constituents:

Acetone—large quantities.
Diacetic acid.
β-oxybutyric acid.
Fatty acids—large quantities.
Fats.
Lactic acid—large quantities.
Cystin.
Leucin.
Indoxyl in large quantities.
Alkapton.
Albuminous substances in considerable quantities.
Blood.
Melanin.
Bile pigment.
Bile acids.

Tyrosin.
Carbohydrates in large quantities.
Phenol in large quantities.
Cresol in large quantities.
Indol-acetic acid in large quantities.
Urobilin—large quantities.
Cholesterin.
Lecithin.
Diamins.
Hydrogen sulphide.

## THE CHLORIDES

The chlorides in the urine are derived from the food and are present in larger amount than all the other inorganic salts combined. The normal elimination of sodium chloride in the twenty-four hours is generally considered from 10 to 15 grams. Steyrer[1] gives 6 to 22 grams as normal limits in healthy persons on a full diet. Lindemann,[2] however, states that he has seen a daily excretion of only 0.8 to 0.9 grams of sodium chloride in healthy men. These low figures, however, are not generally accepted.

If a person is put on a milk diet, or the amount of the mixed diet is reduced, the chlorides are reduced in proportion to the reduction of the intake, but a limit is quickly reached owing to the fact that the tissues and fluids of the body require about 0.5 per cent. of NaCl to keep in osmotic equilibrium.

In normal persons the introduction and retention of large quantities of sodium chloride are accompanied by a rapid increase of several kilos in the body weight due to a corresponding retention of water. If the amount of chlorides is then greatly reduced, the water and the excess of salt will be excreted and the body weight will return to its original amount. Experiments of this type show that even in normal persons there is a marked variability in the amount of water in the body, depending, it seems probable, largely on the sodium chloride content of the tissues.

In starvation the amount of chlorine excreted is very small. The best clinical example of such a condition may be seen in a patient with a carcinoma of the stomach, causing stenosis of the pylorus. Under these circumstances the intake of chlorine in the food is greatly diminished and a portion of that taken in is vomited. The urine in such a case may contain only a few centigrams in 24 hours. The amount of chlorine excreted under these circumstances becomes an index of the amount of food absorbed.

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[1] Beit. z. chem. Phys. u. Path., Bd. ii, 1902, p. 318.
[2] Deut. Arch. f. klin. Med., Bd. lxv, 1899, p. 1.

In general the chlorides in the urine are reduced in febrile diseases, and especially those in which an exudate is formed, such as acute pneumonia, or any condition in which there is an exudate or transudate of any considerable bulk. In the exudate, for instance, of a pleurisy with effusion, there is a large amount of sodium chloride locked up in the fluid; and as the body always needs a certain amount, there is no excess to excrete in the urine. In malaria, however, an increased excretion has been noted during the chill.

In pneumonia, some observers consider that this simple mechanical explanation of the method of the retention of the chlorides is not entirely satisfactory, and Santini,[1] after the examination of a number of cases of acute pneumonia with especial attention to the diminution of the chlorides in the urine, came to the conclusion that the diminution appears shortly after the onset of the fever, it has no prognostic value as to the severity of the disease, and it is not dependent upon the extent of the inflammatory process in the lungs nor upon the height of the fever. The chlorides of the blood rise in proportion to the fall of that substance in the urine, which the writer explains by the assumption that the chlorine enters into combination with nitrogenous substances in the body which prevent its excretion by the kidneys. At the termination of the infection the nitrogenous substances are excreted in the form of urea, and the chlorine set free appears again in the urine in the form of inorganic chlorides.

A severe diarrhœa will also reduce the amount of chlorides in the urine, for all the excess is carried off in the exudation from the bowel. As a pneumonia defervesces or an exudate is absorbed, the excess of the sodium chloride over the needs of the body reappears in the urine and very large amounts may thus be excreted for several days. In both cases the presence of the increased sodium chloride excretion is of prognostic and diagnostic value. A continuous high secretion is seen chiefly in diabetes insipidus.

In nephritis it has been shown [2] that the œdema is largely the result of an attempt of the body to dilute the chlorides which are retained in the tissues owing to three factors: first, the impermeability of the kidney to these salts; second, the sluggish circulation due to failure of the heart; and third, the combination of the chlorides with organic molecules to form a compound of large molecular weight, which renders its excretion difficult. The results of the determination of the conductivity of the blood in nephritis suggest the latter possibility, as no rise is noted, though the number of molecules is increased. Union of the salt with albumin would account for the non-conductivity of some of the retained molecules.

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[1] *Santini:* Riforma Med., 1903, p. 477.

[2] *Widal* et *Lemierre:* Bull. Soc. Méd. des hôpitaux de Paris, 1903; *Widal* et *Javal:* ibid., 1903; also Jour. de Phys. et Path. gén., vol. v, 1903, pp. 1106 and 1123; *Vaquez* et *Laubry:* Bull. Soc. Méd. des hôpitaux de Paris, 1903, p. 1220; *Widal, Fromme* et *Digne:* ibid., 1903, p. 1208.

In nephritis and also in heart disease, with broken compensation and œdema, a diminution of the intake of chlorides assists in the removal of the œdema.

**Qualitative Tests.**—The tests for the chlorides are dependent upon the formation of silver chloride on adding a solution of silver nitrate to a urine previously acidulated with strong nitric acid. This is to prevent the formation of silver phosphate. A 10 per cent. solution of the silver salt is used, and an exactly similar test is to be made on a normal urine as a control. Any reduction in an amount sufficient to be of value in diagnosis can be made out by the difference in the bulk of the precipitate of silver chloride formed in the two test tubes. Albumin must be removed before applying the test.

The qualitative determination of the chlorides in the urine by the above method is usually amply sufficient for the practitioner; but where it is desired to obtain more accurate records of the amount of chlorine excreted, the following simple titration method will suffice to give fairly accurate results.

**Quantitative Determination.**—**Mohr's Method.**—The principle of the test is that the chlorides are precipitated by the addition of silver nitrate solution, using potassium chromate as an indicator. Practically the precipitate consists solely of chloride of silver, the insoluble phosphate of silver being formed only after the production of orange chromate of silver. The strength of the silver solution is conveniently so selected that 1 c.c. corresponds to 1 centigram of sodium chloride. Such a solution contains 29.06 grams of pure fused silver nitrate or 18.449 grams pure silver to the liter, and should be adjusted by titration against a weighed amount of chemically pure and dry sodium chloride.

The technique of the process is as follows:

Ten c.c. of the urine, which must be freed from albumin,[1] are measured out into an Erlenmeyer flask or into a porcelain capsule and 100 c.c. of water are added, and then several drops of potassium chromate solution, sufficient to produce a distinct yellow color. The standard silver nitrate solution is then added from the burette, stirring meanwhile until the reddish orange color which appears first only at the point at which the drop falls into the solution, is distributed throughout the bulk of the fluid. The first permanent trace of orange color is the end reaction. The first titration gives only approximate results, and should be repeated if especial accuracy is necessary. The number of c.c. employed, multiplied by 0.01, gives the sodium chloride.

The results are always too high owing to the other silver compounds which are formed on addition of silver nitrate to the urine, but this error is not of great importance in clinical work.

**Volhard's Method.**[2]—For accurate determination of chlorides in the urine, such as are necessary in experiments on metabolism where the intake and the output of the chlorine can both be determined, the method of Volhard is the most satisfactory.

The solutions necessary are:

1. A decinormal silver nitrate solution;
2. A saturated solution of ammonio-ferric alum;
3. Nitric acid of a specific gravity of 1.2;
4. A decinormal solution of potassium or ammonium sulphocyanide.[3]

Ten c.c. of the urine, which has been freed from albumin, are placed in a 100 c.c. measuring flask, 4 c.c. of nitric acid are added and 5 c.c. of the iron alum solution. If the urine is very dark, three or four drops of a concentrated potassium permanganate solution should be added. On shaking, the color of the fluid becomes pale yellow. An accurately measured amount of silver solution is then added, usually 30 c.c. is sufficient. The flask is accurately filled to the 100 mark with distilled water, shaken thoroughly, the fluid filtered, and 50 c.c. of the filtrate, corresponding to 5 c.c. of urine, are pipetted off. The excess of silver nitrate is determined by titration with the decinormal ammonium sulphocyanide solution until a reddish color appears in the mixture.

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[1] Instead of freeing the urine from albumin, 10 c.c. may be evaporated in a platinum crucible with a small quantity of chlorine-free sodium carbonate and the dried contents of the crucible ashed over a flame at a low heat and the residue extracted with dilute nitric acid and titrated as given above.

[2] Zeit. f. prakt. Chemie, vol. ix, 1874, p. 217; *Salkowski:* Zeit. f. phys. Chemie, Bd. i, 1877, p. 16; also Bd. ii, 1878, p. 397, and Bd. v, 1881, p. 285.

[3] See Appendix for method of preparation of these solutions.

The number of c.c. of sulphocyanide solution used is multiplied by 2 and subtracted from the number of c.c. of silver originally added. The number of c.c. remaining indicates the number of c.c. of silver nitrate solution which have been precipitated from the mixture as a chloride. One c.c. of a decinormal silver solution is equivalent to 0.00355 grams of chlorine or 0.00585 grams of sodium chloride. If, for example, 30 c.c. of $^{N}/_{10}$ silver nitrate were originally used to precipitate the urine and 5 c.c. of sulphocyanide are required to neutralize the silver nitrate, which remains in excess in the 50 c.c. of filtrate used, then 10 c.c. would have been required for the 100 c.c. originally taken. Twenty c.c. of decinormal sulphocyanide solution is equivalent to 20 c.c. of $^{N}/_{10}$ silver nitrate; therefore the 10 c.c. of urine contains twenty times 0.00585 or 0.11700 grams of NaCl, and the total excretion should be determined by multiplying by the number of c.c. passed in twenty-four hours.

While the hydrochloric acid in the urine is combined with all the bases present the results are usually expressed in terms of sodium chloride.

It is generally more convenient to use instead of a decinormal solution of silver nitrate, one which corresponds to an even amount of sodium chloride; as, for example, a solution containing 29.06 grams to the liter, 1 c.c. of which corresponds to 0.01 grams of sodium chloride. In such a case the strength of the sulphocyanide solution must be correspondingly increased.

## THE PHOSPHATES

The phosphates in the urine are, like the chlorides, derived from the food, and undergo considerable fluctuations in normal persons, dependent upon variations in diet. About two-thirds of the phosphoric acid is in combination with the alkalies, sodium, potassium, and ammonium, while the remaining third unites with calcium and magnesium. Traces are found as glycero-phosphoric acid. In chyluria a portion of the phosphorus content of the urine is in the form of lecithin. A portion of the phosphoric acid is derived from the oxidation of the nucleins of the food and of the body tissues, and therefore the excretion of phosphoric acid follows, to a certain extent, that of the purin bodies. This is especially noticeable in pneumonia and in leukæmia, in both of which conditions there is an increased destruction of leucocytes, and, as has been previously stated (see page 6), these cells contain large quantities of nuclein and a phosphorus-bearing substance, lecithin.

The normal excretion in the urine of healthy adults per day may be considered as approximately 2.5 to 3 grams of $P_2O_5$, 5 to 10 decigrams escaping in the fæces. The amount in the urine depends very considerably upon the nature of the food, a larger amount of the phosphoric acid being excreted in the fæces if the food contains much vegetable matter or lime salts. The insoluble calcium phosphate, which is formed under these conditions, is not reabsorbed by the intestine.

The amount of phosphates excreted is reduced in acute infections and in chronic diseases in which the nutrition of the body suffers, though an increased excretion has been noted in the insane.

The phosphates are increased in diabetes mellitus, but it must be remembered that an apparent increase is often due to the large proteid intake of these patients. According to a number of observers the phosphate excretion is increased in the so-called phosphaturia. Patients suffering from this condition pass urine containing a heavy deposit of phosphates, and are usually the subjects of nervous disorders, and frequently of sexual neurasthenia. Whether it is justifiable to speak of the condition as phosphaturia is still doubtful. So far as our present knowledge of the subject goes, these patients do not regularly excrete an increased amount of phosphoric acid. The diagnosis is usually based upon the presence in the urine of a dense sediment of the phosphates of the alkaline earths, but analyses do not show an increased amount of phosphoric acid, merely a diminution in the acidity of the urine, so that Leo[1] has even suggested that the name phosphaturia should be replaced by alkalinuria.

It is far more likely that the nervous condition of the patient incites the phosphaturia than that the phosphaturia is in any way the cause of the nervous symptoms. Even under physiological conditions, a cloudy urine owing to the phosphates is often passed shortly after a heavy meal, especially if the food has been largely proteid in nature so as to require a large excretion of hydrochloric acid for its digestion. The taking of large quantities of alkaline carbonates, or fruit and vegetables which contain organic salts, which can be oxidized into carbonic acid, is frequently followed by the passing of urine containing large amounts of the earthy phosphates. This passes unnoticed in healthy persons, but in neurasthenic patients who examine their own urine, the condition is more frequently brought to the attention of the physician. A good many of these patients suffer from chronic constipation, and in order to remedy this condition eat large quantities of vegetables, which only increases the urinary symptoms. Such patients also are often the subjects of hyperchlorhydria. The increased secretion of hydrochloric acid in the stomach diminishes the general acidity of the blood, and therefore of the urine. When the combination of the acid and digested food is absorbed in the intestine the acidity rises and the urine again becomes acid. If, however, such patients vomit, or have the gastric contents removed by a stomach tube, the alkalinity is more permanent.

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[1] Ueber Alkalinurie, Deut. Arch. f. klin. Med., Bd. lxiii, 1902, p. 604.

An increased deposit of phosphates is also found in the urine in connection with acute or chronic inflammatory conditions of the genito-urinary tract. The alkalinity of the urine is due to ammoniacal fermentation of the urea by bacteria, and the marked increase in the alkalinity causes the precipitation both of the phosphates of the alkaline earths and of ammonium magnesium phosphates. If the urine of such patients is tested for phosphoric acid by titration, the amount will be found quite within the normal limits.

**Quantitative Determination of Total Phosphoric Acid.**—The solutions required are:

1. Uranium nitrate solution of such a strength that 20 c.c. correspond to one-tenth of a gram of phosphoric acid ($P_2O_5$).

2. A solution containing 100 grams of sodium acetate and 100 c.c. of a 30 per cent. solution of acetic acid (sp. gr. 1.04) or 30 c.c. of glacial acetic acid. The mixture is diluted to 1,000 c.c.

3. A tincture of cochineal. This is prepared by digesting at room temperature several grams of cochineal with 250 c.c. of a mixture containing 3 parts of water and 1 part of strong alcohol.

In order to make up the standard solution of uranium nitrate about 45 grams of the salt are weighed out and dissolved in 1100 c.c. of water. To bring the solution to a proper strength it must be titrated against a solution of dihydrogen potassium phosphate. Of this salt, which must be pure and in the form of clear crystals, 3.835 grams are weighed off on an accurate balance, dissolved in water and the solution diluted to a liter. Fifty c.c. of the solution, which contain 0.1 gram of $P_2O_5$, are measured off by a pipette and placed in a beaker and 5 c.c. of the acetic acid mixture are added, then several drops of the tincture of cochineal, and the whole heated just to the boiling point. The uranium nitrate solution is then added until a faint greenish color is noted in the precipitate, which does not disappear when the mixture is stirred. A second titration will give a more accurate result. If the uranium solution is correct, 50 c.c. of the sodium phosphate should require 20 c.c. of the uranium nitrate to produce the end reaction. If less be used the uranium nitrate solution should be correspondingly diluted. If, for example, 19 c.c. are required, it indicates that to each 19 c.c. of the original solution 1 c.c. of distilled water should be added; or, in other words, that 950 c.c. of the original mixture should be diluted up to 1,000 c.c.

To estimate the phosphoric acid in the urine, 50 c.c. of that fluid are mixed in a beaker with 5 c.c. of the acetate mixture, a few drops of the cochineal added and the contents of the beaker heated to boiling and titrated until a faint green color is noted. A second titration is necessary to obtain accurate results. One c.c. of the uranium nitrate solution is equal to 5 milligrams of $P_2O_5$, so that the number of c.c. used, multiplied by 5, gives the milligrams of phosphoric acid in 50 c.c. of the urine titrated.

## THE SULPHATES

The sulphur in the urine is chiefly oxidized and excreted in two forms, known as the preformed sulphates and the combined sulphates.[1] The sulphur in these sulphates is derived chiefly from the oxidation of the proteid material of the food, and about nine-tenths of it combines with the alkalies present in the body to form the preformed sulphates, while the other tenth unites with substances of the aromatic group, chiefly indoxyl, cresol, phenol, and pyrocatechin, to form the conjugate sulphates. The total sulphates excreted depend, therefore, upon the proteid intake; hence they are more abundant in the urine of those eating large quantities of meat, and less so in the urine of persons whose food consists largely of vegetables. The amount in 24 hours is from 2 to 3 grams of $SO_3$. The ratio of 10 to 1 between the preformed and the conjugate sulphates is ordinarily altered by the increase of conjugate sulphates under the following conditions. The decomposition of pus and absorption of the products so formed increases the absolute amount of the ethereal sulphates. Any increase in intestinal putrefaction, whether due to intestinal obstruction or diminution of the flow of bile, will increase the conjugate sulphates. The absence or the increase of the hydrochloric acid of the gastric juice will increase the conjugate sulphates, while diarrhœa will diminish them, as a rule, and simple constipation have but little effect. The amount of the conjugate sulphates is also greatly increased after the administration of drugs containing any of the aromatic groups, such as phenol, cresol, or resorcin, and also in phosphorus poisoning. The ratio between the total nitrogen excreted and the sulphates is fairly constant in health, as both are chiefly derived from the proteid of the food. This ratio is $N : H_2SO_4 = 5 : 1$.

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[1] The latter are also known as the ethereal or conjugate sulphates.

**Tests.**—Simple qualitative tests showing the presence of the sulphates in the urine can be made by adding an excess of barium chloride to a urine previously rendered strongly acid with hydrochloric acid. A cloud shows the presence of a soluble sulphate. If the precipitate is filtered off and the filtrate boiled for a few minutes, a fresh precipitation will be produced by the sulphuric acid released by the hydrochloric acid from combination with the aromatic radicals.

These tests are of no practical value, nor can any conclusions of importance be drawn from the bulk of the sediments deposited during these reactions. The only means of making accurate estimations of the sulphates is by weighing the $BaSO_4$ formed from a given bulk of urine.

**Quantitative Determination of Total Sulphates.**[1]—One hundred c.c. of filtered urine are heated over a flame with 10 c.c. of hydrochloric acid. The mixture should boil gently for about ten minutes, then the flame is removed, and the solution cooled. About 15 c.c. of 10 per cent. barium chloride solution is allowed to drop into the cooled mixture from a burette or a pipette with rubber tube and pinchcock. The flask is not shaken during the process. At the end of an hour the contents are filtered through a Gooch filter (page 750) which is then dried, ignited, and weighed; or if preferred the mixture may be filtered through a small ash-free filter about 9 centimeters in diameter, being careful that all the precipitate is collected on the filter, if necessary by means of a glass rod, with a bit of soft rubber tubing drawn over the lower end. The filtrate must be perfectly clear; if it is not, it should be repeatedly returned through the filter. The clear filtrate is tested by the addition of a few drops of sulphuric acid, to see that there is an excess of barium chloride present. The precipitate is then washed with warm water until the wash water gives no reaction with silver nitrate. The filter is then filled twice with absolute alcohol to remove pigment, especially indigo blue, and once with ether. The filter and its contents are removed from the funnel, folded and wrapped loosely with a platinum wire and held over the mouth of a platinum crucible while the filter paper is ignited by means of a flame and allowed to burn slowly. The precipitate will then fall into the crucible, which should then be heated for a few minutes while still covered, and then for about five minutes longer with the cover off, until the contents are pure white in color. The crucible is allowed to cool in a desiccator and weighed. The difference between the original weight of the crucible and the weight of the crucible plus the precipitate, gives the amount of barium sulphate. The amount of sulphuric acid ($H_2SO_4$) is obtained by multiplying the weight of $BaSO_4$ by 0.42, that of $SO_3$ by 0.3429.

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[1] *Salkowski:* Practicum, second edition, p. 263.

**Quantitative Determination of Ethereal Sulphates.**—One hundred c.c. of urine are mixed with 100 c.c. of an alkaline barium chloride solution containing two volumes of baryta water and one volume of 10 per cent. barium chloride solution. The whole is stirred and filtered after a few minutes through dry filter paper into a dry beaker. One hundred c.c. of the clear filtrate is measured off, acidified slightly with hydrochloric acid, then 10 c.c. of concentrated hydrochloric acid are added and the determination carried out as in the previous paragraph for the determination of the total sulphates. In this case the preformed sulphates are removed by the addition of the barium chloride mixture, leaving the ethereal sulphates in solution. These are then broken up by the heating with strong hydrochloric acid and the acid unites with the barium salt to form $BaSO_4$. The weight of the barium sulphate, multiplied by 0.3429, gives the $SO_3$. This subtracted from the results obtained from the determination of the total sulphates gives the amount of the preformed sulphates.

### NEUTRAL SULPHUR

Another form of sulphur appears in the urine contained in the sulphocyanides, taurin derivatives, etc., but chiefly in the oxyproteic acids. It may form 10 to 30 per cent. of the total sulphur in the urine. The amount is increased in starvation, narcosis, and poisoning, and in pneumonia and jaundice. This latter phenomenon is due to absorption of sulphur compounds from the bile. In cases of cystinuria the neutral sulphur may be greatly increased. The study of neutral sulphur excretion has as yet furnished no facts of clinical importance.

There is no satisfactory method for determining neutral sulphur direct; but the total sulphur in the urine can be determined and the amount of neutral sulphur computed by subtracting from the total the preformed and ethereal sulphates.

**Determination of Neutral Sulphur (Benedict).**—Ten c.c. of urine are measured into a porcelain evaporating dish, 7 or 8 cm. in diameter, and 5 c.c. of reagent added, composed of crystallized copper nitrate, 200 grams, sodium or potassium chlorate, 50 grams, and distilled water to 1,000 c.c. The contents of the dish are evaporated over a free flame which is regulated to keep the solution just below the boiling point. When dryness is reached the flame is raised slightly until the entire residue has blackened. The flame is then turned up in two stages to the full heat of the Bunsen burner and the contents of the dish heated to redness for ten minutes after the black residue (which first fuses) has become dry. This heating decomposes the last traces of nitrate and chlorate. The flame is removed and the dish allowed to cool. Ten to twenty c.c. of dilute (1 to 4) hydrochloric acid is then added to the residue in the dish, which is warmed gently until the contents have completely dissolved and a perfectly clear, sparkling solution is obtained. This dissolving of the residue requires scarcely two minutes. The solution is washed into a small Erlenmeyer flask, diluted with cold distilled water to 100 to 150 c.c., 10 or 20 c.c. of 10 per cent. barium chloride solution added drop by drop, and the solution allowed to stand for about an hour. It is then shaken up and filtered as usual through a weighed Gooch crucible or ash-free filter paper.

## THE OXALATES

From 10 to 20 milligrams of oxalic acid per day are excreted in the urine under ordinary circumstances, though the amount may occasionally reach 50 milligrams. A portion of the oxalates in the urine is derived from the food, while the remainder is formed in the body by a little-understood process of metabolism. Not all, however, of the oxalic acid taken in in the food is excreted as such. The greater portion is probably destroyed by the action of the intestinal bacteria, for a number of the commonly used articles of diet[1] contain as high as 0.3 per cent. of oxalic acid, so that a very few grams of such food would furnish the amount found in the urine. A considerable portion of the acid ingested escapes in the fæces in the form of calcium oxalate.

Calcium oxalate crystals are often found in the urine of persons suffering from gastro-intestinal disturbances, sometimes in connection with increase of indoxyl in the urine, occasionally with no increase in that substance but with exaggerated excretion of the aromatic sulphates.

The older writers laid great stress on the clinical symptoms of this so-called oxaluria. Among these symptoms were headache, lassitude, mental depression, digestive disturbances, loss of appetite, flatulence, constipation, etc. Recent studies of the excretion of oxalic acid in disease have not confirmed these views, which were based largely upon the connection of these symptoms with the appearance of calcium oxalate in the urine.

It has been shown [2] that urine in which a noticeable number of calcium oxalate crystals can be demonstrated microscopically, contains about 25 milligrams of oxalic acid to the liter, that is a practically normal amount, while those urines which show no such deposit rarely contain more than 10 milligrams. In other words, abundant crystals can appear in the urine without an excessive excretion of oxalic acid. Fürbringer [3] has also called attention to the fact that a specimen of urine may contain a large amount of oxalic acid in soluble form without a sediment of calcium oxalate crystals being formed on standing.

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[1] Among these foods are spinach, rhubarb, figs, cocoa, tea, coffee, pepper, green peas, plums, tomatoes, and strawberries.

[2] *Dunlop :* Jour. of Path. and Bacteriology, 1896, vol. iii, p. 389.

[3] Deut. Arch. f. klin. Med., Bd. xviii, 1876, p. 143.

Baldwin[1] has been able to develop an experimental oxaluria in animals by feeding large quantities of glucose together with meat. This experimental oxaluria is associated with mucous gastritis and absence of hydrochloric acid in the gastric contents. The oxalic acid is formed by fermentation of the carbohydrates in the stomach. The symptoms usually considered as due to oxaluria are rather an expression of poisoning due to the absorption of putrefactive products from the intestine.

Large quantities of oxalic acid are occasionally passed by diabetic and obese persons, but only as a rare phenomenon,[2] the pathology of which is as yet unknown.

The chief interest connected with the presence of calcium oxalate crystals in the urine is, therefore, not that they show presence of a disordered metabolism, but in the possibility that such crystals may form calculi in some portion of the genito-urinary tract.

**Quantitative Determination of Oxalic Acid.**[3]—Five hundred c.c. of unfiltered urine are evaporated over a small flame to about 150 c.c. Twenty c.c. of concentrated HCl are added and the mixture is shaken out three times, using 200 c.c. of ether, containing 10 per cent. of alcohol. The 600 c.c. of the ethereal extract are carefully separated from the aqueous residue and filtered through dry paper into a dry flask. The ether is distilled off and the residue poured into a porcelain dish. The flask is rinsed out with alcohol and water and these washings added to the contents of the dish. Some water is added and the whole warmed over a bath until no odor of ether or alcohol can be noticed. The remaining fluid (about 20 c.c.) is filtered, rendered slightly alkaline with ammonia, and 2 c.c. of a 10 per cent. calcium chloride solution are added. Acetic acid is then added to a faint acid reaction. Calcium oxalate separates out and is collected on a small, ash-free filter, washed with water, dried and strongly ignited. The weight of the CaO obtained, multiplied by 1.6, gives the amount of oxalic acid. The residue of CaO should not give off $CO_2$ when dissolved in HCl, nor should a precipitate be produced by ammonium molybdate (phosphoric acid).

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[1] Jour. of Experimental Medicine, vol. v, 1900, p. 27.

[2] *Naunyn:* Diabetes Mellitus, Nothnagel's Handbuch d. Spec. Path., Bd. vii, p. 174, Wien, 1898; *Mohr* u. *Salomon*, Deut. Arch. f. klin. Med., Bd. lxx, 1901, p. 486.

[3] *Salkowski:* Practicum, p. 254.

Autenrieth and Barth[1] have suggested precipitating the oxalic acid directly in the fresh urine by an excess of $CaCl_2$ and $NH_3$, and after allowing the mixture to stand for twenty hours, collecting and dissolving the precipitate in concentrated HCl. This solution is then to be shaken out with ether as above.

Baldwin has found that a somewhat simpler method gives satisfactory results. Five hundred c.c. of the twenty-four hours' specimen are mixed with 150 c.c. of 95 per cent. alcohol in order to precipitate the calcium oxalate. The mixture is set aside for forty-eight hours, and then filtered, care being taken to remove the crystals from the walls of the beaker by a rod, the tip of which is protected by a rubber tube. The sediment is washed thoroughly with hot water and 1 per cent. acetic acid. The filter is then placed in a small beaker and soaked in a few c.c. of dilute hydrochloric acid. The filter is then washed with hot water until there is no further acid reaction, and the washings are collected and evaporated to 20 c.c. A little calcium chloride solution is added to insure an excess of calcium. The hydrochloric acid is neutralized with ammonia, and then the solution is rendered slightly acid with acetic acid. Strong alcohol is added in an amount equal to one-half the volume of the fluid, and the whole is set aside for another forty-eight hours. The sediment of calcium oxalate is collected on an ash-free filter, washed with cold water and 1 per cent. acetic acid until free from chlorides, the filter is incinerated, cooled and weighed. The ash is calcium oxide, each gram of which represents 1.6 gram of oxalic acid.

### AMMONIA

The urine of an adult in good health and on a mixed diet contains from 0.6 to 1.2 grams of ammonia in twenty-four hours. The ratio between the total nitrogen and the nitrogen derived from the urinary ammonia is fairly constant on a mixed diet, and is about one hundred to five or six. Both factors are increased when the diet is largely composed of meat, and diminished with a purely vegetable diet, but these changes are slight and variable. A moderate amount of fat does not change the $NH_3$ ratio, but large amounts cause a marked increase. The action is probably due to the large amount of acid split off from the fats, which combines with the ammonia of the body and thus increases the $NH_3$ in the urine. At the same time the acetone of the urine is increased and even diacetic or $\beta$-oxybutyric acids may be present.[1] As soon as sufficient carbohydrate is added to the food the abnormal acids disappear from the urine.

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[1] Zeit. f. phys. Chem., Bd. xxxv, 1902, p. 327.

An evidence of the correctness of the theory that an excessive acid production is at the base of this phenomenon is the fact that a marked increase in the ammonia ratio can be induced much more easily in chronic liver diseases by the administration of large quantities of fat than is possible in normal persons.[2]

In acute gastro-enteritis, especially in children, where there is an increased acidity of the tissues and the acetone group of substances are found in the urine, the ammonia nitrogen factor is greatly increased, rising occasionally to 40 per cent. of the total N. In acute infectious diseases, also, the ammonia is often increased.

The increase in the $NH_3$ ratio is noted, however, much sooner than the appearance of increased amounts of acetone. The same conditions are noted in diabetes, when the body fluids contain an excess of acids which have been shown to be derived from the fat of the tissues or the food.[3] The daily excretion under these conditions may reach 12 grams of $NH_3$.

The ammonia ratio is reduced when the secretion of HCl in the stomach is reduced, while it is increased in cases of hypersecretion, but this is true only in persons in approximately normal conditions, for other factors enter when the metabolism of the organism is greatly disturbed. For example, the ammonia ratio is high in starvation and in benign or malignant stenosis of the pylorus, though in this case the HCl secretion may be diminished. Large doses of fixed alkalies diminish the ammonia ratio.

In diseases of the liver, especially cirrhosis, an increase in the ammonia ratio is often, though not always, observed, which in some cases seems to be due to interference with the normal process of urea synthesis, and in others to a moderate acidosis of the fluids of the body, for the $NH_3$ can be reduced to normal limits by the exhibition of large doses of alkalies.

**Quantitative Determination of the Ammonia in the Urine.**—*Method of Nencki-Steyrer*[4].—Twenty to thirty c.c. of the perfectly fresh urine, depending upon its concentration, are placed in the flask *A* (Fig. 133). The flask is then closed by a double perforated cork carrying a dropping funnel, *T*, and a long tube, *C*, which is drawn out at its lower end to a fine opening. *C* is connected with a wash bottle containing sulphuric acid, and the stream of air which flows through is regulated by the cock *R*. In the flask *B* is placed one-fourth normal sulphuric acid, which is kept cool with ice-water. Tube *E* is so bent that it reaches the bottom of the flask and is covered by the acid. At *D* it is joined with a tube from the flask *A* by means of a heavy, tightly fitting rubber cylinder. The bulb *S* is used to prevent any spurting over of the sulphuric acid. *S* may be directly connected with the water pump, or, as shown in the figure, may run into a Woulfe bottle to collect any acid which may spurt over. Fifty c.c. of milk of lime is allowed to run from the funnel into the urine in flask *A*. *T* is immediately closed and the pump is started. *R* is then so regulated that while some air is admitted, yet the vacuum remains at from 18 to 25 mm. of mercury. *A* is then warmed with a water bath to about 36° C. At this temperature and pressure the fluid soon boils, and any bumping is prevented by the air stream through the tube *C*. In the course of an hour all the ammonia is distilled into the acid in flask *B*. As soon as the distillation is completed the bulb *S* is washed out into *B* with distilled water and the acid titrated with one-fourth normal sodium hydrate to determine the amount used. Some alcohol should be mixed with the milk of lime when the urine contains albumin, as foaming is to a certain extent prevented by this admixture.

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[1] *Gerhardt* u. *Schlesinger:* Arch. f. exp. Path. u. Pharm., Bd. xlii, 1899, p. 107.

[2] *Schittenhelm:* Deut. Arch. f. klin. Med., Bd. lxxvii, 1903, p. 517.

[3] *Magnus-Levy:* Arch. f. exp. Path. u. Pharm., Bd. xlv, 1901, p. 389.

[4] *Steyrer:* Beit. zur chem. Phys. u. Path., Bd. ii, 1902, p. 312.

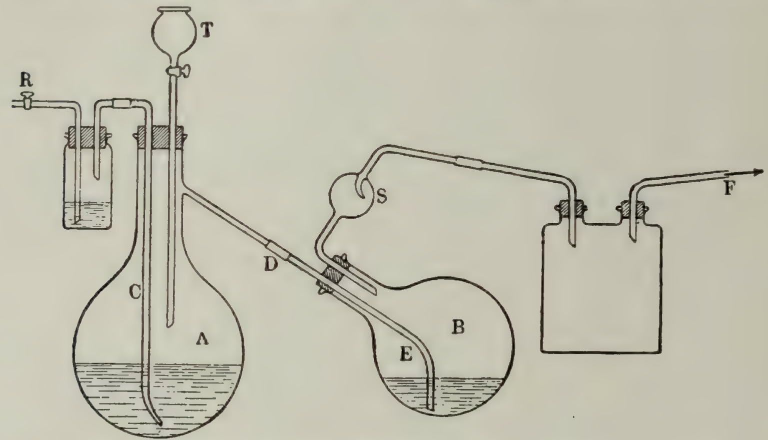

FIG. 133.—APPARATUS FOR THE DETERMINATION OF AMMONIA. (Steyrer.)

*Folin Method.*[1]—The ammonia is set free by the addition of a weak alkali (sodic carbonate), then removed from the urine at room temperature by means of a strong air current, collected in decinormal acid, and titrated.

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[1] *Folin:* Zeit. f. phys. Chemie, Bd. xxxvii, 1902, p. 161; Amer. Jour. of Phys., vol. xiii, 1905, p. 47.

Twenty-five c.c. of urine is measured into an aërometer cylinder (30 to 45 cm. high), and about a gram of dry sodic carbonate and some kerosene or fluid albolene are added to prevent foaming. The upper end of the cylinder is then closed by means of a doubly perforated rubber stopper, through which pass two glass tubes, only one of which is long enough to reach below the surface of the liquid. The shorter tube, about 10 cm. long, is connected with a "calcium chloride tube" filled with cotton to catch any alkaline spray. This tube in turn is connected with a glass tube extending to the bottom of a wide-mouth bottle of about 500 c.c. capacity, containing 20 c.c. decinormal acid, 200 c.c. of water, and 2 drops of a 1 per cent. solution of alizarin red as an indicator. The complete absorption of the ammonia, which is set free from the urine and combines with the acid, is most easily insured by the use of an absorption tube, which compels a very intimate contact of the air coming from the cylinder with the acid and water in the absorption bottle. This absorption tube consists of a glass tube with a small perforated bulb at its lower end, which is inserted by means of a rubber cork into a cylindrical glass tube measuring about 2.5 cm. in diameter, which is open at its lower end and is perforated with a series of openings about 3 cm. from its upper end where the cork is inserted.[1]

The air passing first through the alkaline urine, and then through the standard acid, transfers every trace of ammonia in the course of a relatively short time (1½ hours) from the urine to the acid, and the ammonia can then be determined by direct titration. The end point is the first red tint, and not the violet color produced by the addition of a considerable quantity of alkali. The air current must be rapid, that is, several hundred liters per hour. An ordinary good filter pump is sufficient if the water pressure is not too low; otherwise some form of blast pump must be used. A control should always be made when the apparatus is first set up, to see whether sufficient air passes, by replacing the acid in the absorption chamber with fresh, and then allowing the apparatus to run for several hours longer. The escape of further quantities of ammonia can be determined by titration. If any has been carried over, it shows that the air current is not sufficient and the process must be continued until no additional ammonia can be removed.

It has been shown [2] that this method does not give the entire amount of ammonia present in the urine if crystals of ammonio-magnesium phosphate separate during the aeration of the fluid. Gies [3] has found that if instead of using sodium carbonate, the 25 c.c. of urine are mixed with 1 gram of sodium hydrate and 15 grams of sodium chloride, the ammonia will be removed by the air current even from the ammonio-magnesium salt.

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[1] These absorption tubes can be obtained from Eimer & Amend, New York.

[2] *Steel* and *Gies*: Jour. of Biological Chem., vol. v, 1908, p. 71.

[3] Personal communication.

## IRON IN THE URINE

The iron present in the food or administered therapeutically is absorbed from the intestine. A very small portion reappears in the urine, and traces are excreted in the bile; while the surplus is reëxcreted into the intestinal lumen and passes off in the fæces. Under normal conditions of a mixed diet the bile contains about 1 milligram of iron per day, the fæces 8 to 10 milligrams and the urine 1 milligram. In chlorosis the iron in the urine may rise to 2.7 milligrams. In pernicious anæmia eight or nine times the normal amount may be excreted. The amount is also increased in fever, nephritis, diseases of the liver, leukæmia, and chronic alcoholism. A great increase in the amount has been noted in diabetes, but not in all cases.

The methods of estimating the iron in the urine are exceedingly complicated. Colorimetric procedures have been described by Hueck[1] and by Marriott and Wolf.[2] That of Neumann[3] is considered the most accurate.

**Quantitative Determination of Iron in the Urine.** (Method of Neumann.)—The reagents required are:

1. A standard solution of ferric chloride which contains 2 centigrams of iron and 2 c.c. of concentrated hydrochloric acid (sp. gr. 1.19) to the liter. The solution is made by dissolving iron wire in the acid, and can be preserved unchanged for a long time in a dark-colored bottle.

2. Thiosulphate solution containing 1 gram of sodium thiosulphate and 1 gram of ammonium carbonate dissolved in a liter of water. The solution should be kept in a brown bottle.

3. Starch solution, made up by dissolving 1 gram of starch in half a liter of boiling water. The heating should be continued for about ten minutes in order to complete the solution of the starch.

4. Zinc reagent, which is made up by dissolving 25 grams of iron-free zinc sulphate and 100 grams of sodium phosphate in separate amounts of water and mixing the solutions in a liter measuring flask. The pre-

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[1] Inaug.-Diss., Rostock, 1905.

[2] Jour. Biological Chemistry, vol. i, 1906, p. 451. For further details see *Damaskin:* Arb. d. pharm. Institut, Dorpat, Bd. vii, 1891, p. 40; *Mayer:* Zeit. f. klin. Med., Bd. xlix, 1903, p. 475.

[3] Zeit. f. phys. Chemie, Bd. xxxvii, 1903, p. 115.

cipitate of zinc phosphate is just dissolved by the cautious addition of dilute sulphuric acid, and the solution should be filled up to a liter.

Blank experiments should be carried out with all these solutions in order to determine whether they are free from iron.

Inasmuch as the thiosulphate solution does not keep, its titer must be determined quite frequently, in the following manner.

Ten c.c. of the ferric chloride solution is mixed in a flask with some water, a few c.c. of starch solution, and about a gram of potassium iodide. The whole is warmed to about 60° C., and titrated with the thiosulphate solution until the blue color passes into violet, and then just disappears. The number of c.c. of thiosulphate solution corresponds then exactly with 2 milligrams of iron. In a few minutes the solution again shows a faint violet color.

Five hundred c.c. of the urine to be tested is mixed with 50 c.c. of concentrated sulphuric acid and the acidified urine allowed to fall drop by drop into a Jena flask, containing 30 c.c. of concentrated nitric acid. The addition of the urine should take place at such a speed that when the fluid is boiling strongly the volume in the flask does not exceed 100 c.c. The whole mixture is concentrated to 50 c.c. and mixed with 10 c.c. of equal parts of concentrated sulphuric acid and nitric acid. The whole is gently heated until the fluid shows merely a faint yellow color. Three volumes of water are added and the whole is boiled for about ten minutes, a brownish vapor being given off on decomposition of the nitrosyl sulphuric acid. The solution is then mixed with 20 c.c. of the zinc reagent, and, after cooling, with ammonia until the white precipitate which falls is permanent. A slight excess of ammonia is added to dissolve the precipitate and the fluid heated to the boiling point. The crystalline deposit which may be formed is separated from the fluid by decantation, and the hot fluid is passed through a small filter, and the filtrate tested with hydrochloric acid and potassium sulphocyanide. If a strong red color is produced, the filtrate must be poured back into the flask and again heated. If the amount of iron is very small, 10 c.c. of the standard iron solution should be added and afterwards 2 milligrams of iron subtracted from the final result.

The precipitate in the flask is now washed three times with hot water and the excess decanted, the wash water being tested by the addition of a crystal of potassium iodide, some starch solution and a drop of hydrochloric acid, when no violet should be produced,—an evidence that no nitrous acid is present. The precipitate is then transferred to a flask and dissolved in a small quantity of hot dilute hydrochloric acid. It is then ready for titration with the thiosulphate mixture in exactly the same manner as is given above for standardizing the thiosulphate solution.

## AROMATIC BODIES

### PHENOL AND CRESOL

These two bodies are excreted in the urine as ethereal or conjugate sulphates and glycuronates, and give practically the same reactions. They are increased under much the same conditions as indoxyl, although the excretion may not run parallel to that of the indoxyl. The clinical importance of the tests for phenol are chiefly in connection with absorption from carbolized dressings or in detecting poisoning due to the taking of phenol by mouth, either in dilute form or in drugs, such as salol, which contain phenol.

**Tests.**—To test for phenol in the urine add strong nitric acid and boil. The urine will give an odor of bitter almond oil. After the contents of the test tube have cooled, the addition of bromine water will cause the formation of a precipitate of tribromnitrophenol. If a portion of the original test be rendered strongly alkaline with NaOH, the color will become an orange red from the formation of the sodium compound of nitrophenol. For other tests acidulate 200 c.c. of urine with 50 c.c. strong hydrochloric acid and distil. When 50 c.c. have passed over, apply the following tests to the distillate.

*a.* On the addition of a small quantity of ferric chloride to the distillate the solution becomes a deep amethyst blue color. This same reaction is given by many phenol derivatives—for example, salicylic acid.

*b.* Heating the distillate with Millon's reagent gives a red color if phenol is present.

*c.* The addition of bromine water causes the formation of a gelatinous precipitate of monobromphenol, which has a very penetrating odor; further addition of bromine causes the formation of a yellowish-white precipitate of tribromphenol. The latter reaction can be used for quantitative purposes.

**Quantitative Estimation of Phenol.**—Two hundred c.c. of urine are acidulated with 25 c.c. of sulphuric acid and distilled until 100 c.c. have passed over. Bromine water is added and the precipitate is allowed to stand for forty-eight hours, collected on a filter, dried in the dark, and weighed. The weight obtained multiplied by 0.283 gives the amount of phenol and cresol present in terms of phenol.

**Quantitative Determination of Phenol according to Kossler and Penny.**[1] —Five hundred c.c. of urine are evaporated to about 100 c.c., great care being given to keep the reaction alkaline. The concentrated urine is transferred to a distillation flask, 25 c.c. of sulphuric acid are added and the contents are distilled. As soon as the fluid in the flask begins to bump, it should be diluted with distilled water and the distillation repeated. The process should be repeated five or six times. The distillate is thoroughly shaken up with calcium carbonate, in order to neutralize the formic and nitrous acids present, and the fluid again distilled. This second distillation should be repeated several times.

The distillate is then collected in a large flask. A solution containing 1 gram of sodium hydrate and 6 grams of lead acetate is added, and the whole heated for about fifteen minutes on a water bath, and finally boiled with an ascending condenser until a few c.c. of the distillate will not reduce ammoniacal silver solutions. This ordinarily requires about five minutes. Longer heating is to be avoided.

Phenol remains as a basic lead phenate, while the other substances which are capable of reacting with iodine pass off. The contents of the flask are then strongly acidified with dilute sulphuric acid and the phenol is distilled off, replacing the water twice. An aliquot part of the measured distillate is treated with decinormal sodium hydrate containing no nitrites until a strong alkaline reaction is obtained. The fluid is then warmed in hot water and a decinormal iodine solution is added, usually about 15 to 25 c.c. more than the number of c.c. of sodium hydrate used. The flask is then stoppered and shaken thoroughly. After the mixture is cooled it is again acidified and the free iodine is retitrated with a decinormal sodium thiosulphate solution, until the color changes from violet to red, using starch paste as an indicator. One c.c. of decinormal iodine solution corresponds to 1.567 milligrams of phenol or 1.802 milligrams of cresol.

## INDOL

Indol, when absorbed in the circulation, is oxidized and excreted in the urine in the form of the potassium salt of indoxyl sulphate, and the tests for this substance are given under the heading of indoxyl. A small amount of the indoxyl may appear as a conjugate compound with glycuronic acid. The tests for indol in substance are chiefly of use in examining urine for possible fæcal contaminations, such as may occur with a recto-vesical fistula.

1. Indol gives the Legal's reaction. When a solution is treated with a few drops of sodium nitroprusside and a little NaOH, a deep violet color is produced, and when acidulated with

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[1] Zeit. f. phys. Chemie, Bd. xvii, 1893, p. 117; also, Bd. xxvii, 1899, p. 123.

acetic acid the color changes to an azure blue. The reaction gives about one part in 50,000 of indol.

2. Cholera-red reaction. The fluid containing indol is mixed with a few drops of very dilute sodium nitrite solution and layered over sulphuric acid. A purple color is produced at the point of contact, and a diffuse pink on shaking the contents of the test tube. If the fluid is neutralized with NaOH the color changes to a blue-green.

3. If a cold saturated watery solution is acidulated with nitric acid and a few drops of a freshly prepared sodium nitrite solution, there is formed a brick-red precipitate of nitrosoindol. Very dilute solutions show only a faint red, but if they are shaken out with chloroform a red layer will separate at the point of contact of the chloroform with the watery solution. The test shows one part of indol in 200,000.

4. Aldehyde reaction.[1] The urine is rendered strongly alkaline and distilled. To 10 c.c. of the distillate is added 1 c.c. of a solution of para-dimethyl-amido-benzaldehyde. This solution is made by dissolving 15 grams of the aldehyde in a mixture of 270 c.c. of water and 30 c.c. of concentrated sulphuric acid. One part of indol in 300,000 gives a cherry red color, which can be extracted by amyl alcohol and shows an absorption band to the green side of *D*. Skatol gives a blue color to chloroform and no band.

### SKATOL

Skatol, after absorption from the intestine, is excreted in the urine as indol-acetic acid, and possibly also as indoxyl[2] under the same conditions as those governing the presence of indol.

**Tests for Skatol.**—1. The urine or distillate is rendered slightly alkaline with sodium carbonate and shaken out with thiophene-free benzol. A measured portion of the benzol is diluted with one-fifth volume of a 5 per cent. solution of p-dimethylamidobenzaldehyde. The mixture is layered over a small quantity of concentrated hydrochloric acid. If skatol is present a bluish violet color appears at the point of contact. Or 1 c.c. of an acid solution of benzaldehyde made by dissolving 5 grams in 100 c.c. of 10 per cent. sulphuric acid may be added to 5 c.c. of distillate.

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[1] *Ehrlich:* Med. Woche, 1901, p. 151; *Herter:* Jour. Biol. Chem., vol. i, 1906, p. 251; ibid., vol. ii, 1906, p. 10.

[2] *Maillard:* L'Indoxyl Urinaire, Paris, 1903.

2. *Sasaki's Method.*—About 3 c.c. of the skatol solution to be tested are mixed with 3 drops of Kahlbaum's aldehyde-free methyl alcohol, and the mixture is layered over an equal volume of concentrated sulphuric acid. A reddish violet ring appears when the skatol is in a concentration of 1 to 50,000. If the fluid is shaken after standing, 1 part of skatol to 5,000,000 gives a reddish violet throughout. The sulphuric acid must contain a trace of ferric salt. This is usually present in the commercial acid, but if not, 1 drop of a 1 per cent. solution of ferric sulphate to 100 grams of pure concentrated sulphuric acid will be sufficient.

**Tests for Indol-acetic Acid.**—The urine is mixed with an equal volume of concentrated hydrochloric acid and a few drops of a .1 per cent. solution of sodium nitrite. A reddish or purplish color appears throughout the fluid. This should be shaken out with amyl alcohol and tested spectroscopically. A band appears in the green close to the D line, corresponding to a wave length of 577 to 555. The coloring matter is not soluble in chloroform.

A reddish color not infrequently appears in urines, which does not give the characteristic absorption band, and a number of urorosein pigments have been described. But the exact relationship between these pigments and indol-acetic acid, if there be any, has not been definitely determined.

Indol-acetic acid appears in the urine in intestinal putrefaction with the production of much skatol, and Herter thought that the cases showing indol-acetic are more difficult to cure than those showing indoxyl only. It has been noted in tuberculous ulceration of the intestine, in cases of carcinoma of the stomach and intestine, and in a number of acute infectious diseases. Large quantities have also been found in the urine in certain types of anæmia, especially of the pernicious group. It is also frequently found in considerable quantities in the urine of infants suffering from chronic intestinal disorders, a clinical aspect of which has been described by Herter under the name "infantilism."

### INDOXYL (INDICAN)

The action of the intestinal bacteria upon the proteids of the food results in the formation of indol, a fact which can be easily verified by testing an extract of the fæces or a broth culture of the colon bacillus. Indol is also produced by the putrefaction of the proteids which goes on in the fluid of an empyema or the cavity of a gangrenous lung. The indol so formed is absorbed by the blood and oxidized, possibly in the liver,[1] to indoxyl, which combines with sulphuric acid to form the so-called ethereal or conjugate sulphates, and in this form is excreted in the urine.

Not all the indoxyl is produced by bacterial action, but a small quantity is probably formed during the breaking down of the body proteids, especially during fever or sepsis.[2]

It is present in small quantities, usually not over 15 milligrams, in the urine of healthy adults, but those who consume large quantities of meat may excrete 60 milligrams. It appears in increased quantities in any condition which permits an increased putrefaction in the intestines and aids in the absorption of the indican so formed; hence its great abundance in the urine of patients suffering from intestinal obstruction, whether due to mechanical constriction of the gut or to the paralysis of the bowel which accompanies acute peritonitis.

It is also present in the urine of typhoid fever patients, especially after a hæmorrhage or perforation of the intestines, and in persons suffering from any form of gastro-enteritis. It is increased in the so-called intestinal indigestion, or "biliousness," and moderate amounts appear in the urine when the secretion of hydrochloric acid is either diminished or increased in the stomach by disorders of that organ. A slight increase over the normal may be noted in many conditions with which it seems to have no symptomatic connection, and merely indicates a slightly increased putrefaction in the lumen of the small intestine.

Obstruction of the colon near its lower end, or simple constipation, does not noticeably increase the amount of indoxyl excreted. Cases have been seen where no movements have taken place for three or four days without marked indoxyluria being produced.

The diagnostic value of an increased excretion of indoxyl is seen chiefly in connection with the diagnosis and the treatment of the milder forms of intestinal disturbance, which are functional rather than organic, and which make themselves known by the symptoms classed together under the expression "biliousness." It has been found experimentally that part, at least, of the lassitude and headache which accompany this condition can be produced by the administration of small doses of indol or indoxyl to healthy persons.

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[1] *Hervieux :* Jour. de Phys. et Path. gén., vol. vi, 1904, p. 426.

[2] *Blumenthal* u. *Rosenfeld :* Charité Annalen, Bd. xxvii, 1903, p. 46. See, however, *Scholz* (Zeit. f. phys. Chemie, Bd. xxxviii, 1903, p. 513), who has been unable to find an increase in indican due to breaking down of the body tissues.

Calculi made up of indigo have been described,[1] and occasionally in the sediment of urine undergoing alkaline fermentation small masses of indigo may be found. These usually result from the breaking up of a compound of indoxyl, in which glycuronic acid takes the place of the sulphuric. This is present in very small quantities, and is much more easily decomposed than the indoxyl-sulphuric acid during the alkaline decomposition of the urine. In the Obermayer's test this compound is also broken up and the indigo formed reacts with that due to the sulphuric acid body.

### *Qualitative Tests for Indoxyl*

The tests for indoxyl are based upon the decomposition of the sodium or potassium compound of the indoxyl-sulphuric acid present in the urine by strong hydrochloric acid and oxidizing the indoxyl thus set free by a suitable reagent.

The combination usually selected is that known as Obermayer's reagent, and is composed of strong hydrochloric acid of a specific gravity of 1.19, to which has been added two parts to the thousand of ferric chloride. This forms a fuming yellow liquid, which keeps indefinitely.

The urine is preferably precipitated with a small amount of a solution of lead acetate or subacetate, avoiding an excess, and filtered. This removes the pigment, which otherwise forms an emulsion with the chloroform used to extract the indigo from the mixture, and prevents the recognition of the blue color produced in the chloroform by the dissolved indigo.

Fifteen c.c. of the filtered urine are mixed with an equal bulk of Obermayer's reagent and about 2 c.c. of chloroform is added. The tube is then corked or the finger is protected by means of a small piece of sheet rubber or a rubber cap, and the vessel containing the mixture *slowly inverted,* not violently shaken.

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[1] *Ord:* Berl. klin. Woch., 1878, p. 365. *Chiari:* Prag. med. Woch., Bd. xiii, 1888, p. 541. *Weber:* Lancet, vol. ii, 1901, p. 774. *Stockman:* Edinb. Med. Jour., vol. xii, 1902, p. 115. (Both of these papers are largely devoted to the differential tests between indigo blue and methylene blue when excreted in the urine.)

Usually the chloroform will immediately take on a dark blue, which is deeper in proportion to the amount of indoxyl present, but it may be necessary to allow the whole to stand for a short time to allow for the complete oxidation of the indoxyl by the ferric chloride.

Normal urine gives a very faint blue by this method; any increase is indicated by a deep blue tint in the chloroform.

Other oxidizing agents may be used, such as hydrogen peroxide, ammonium persulphate, or a strong solution of bleaching powder, or a very small quantity of strong nitric acid, first mixing the urine with an equal bulk of HCl; but with all these reagents great care is necessary to avoid overoxidation of the indigo and the production of indigo red, in which case the presence of indoxyl may be entirely overlooked.

After large doses of codeine a purple-red color is often observed during the course of the test which obscures the blue of the indoxyl.

If the urine contains iodine, the chloroform will be colored an intense carmine, and the blue due to small quantities of indoxyl will be entirely obscured. In this case it is necessary to add to the test tube, after the reaction has been obtained and the excess of hydrochloric acid decanted and replaced by water, a small quantity of a strong solution of sodium thiosulphate (hyposulphite). The tube is again shaken up, and when the chloroform has again settled to the bottom of the mixture, the red due to the iodine will have disappeared and the blue of the indigo be easily seen.

Another method is to decant the chloroform extract and shake it up with dilute potassium hydrate, when the iodine will be removed and the pure blue of the indigo will appear.

### *Quantitative Estimation of Indoxyl*

**Strauss's Method.**—A quantitative method of determining indican, which can be used clinically, has been devised by Strauss.[1] Twenty c.c. of the urine are mixed with 5 c.c. of a 20 per cent. lead acetate solution and filtered. Ten c.c. of the filtrate, corresponding to 8 c.c. of the urine taken, are placed in a small graduated separatory funnel and mixed with 10 c.c. of Obermayer's reagent.

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[1] Deut. med. Woch., 1902, p. 299.

Five c.c. of chloroform are then added and the tube corked and gently shaken. The shaking is repeated in about two minutes and the chloroform allowed to escape from the tube. Another 5 c.c. of chloroform are added and the extraction repeated. This is continued until the chloroform is no longer colored blue by the indigo. Two c.c. of the united chloroform extracts are placed in a small test tube, which is exactly of the diameter of the test tube containing the standard solution, and chloroform is added, drop by drop, until the color of the chloroform extract is exactly that of the control tube, when both are compared against a white background. The standard solution is prepared by dissolving 1 milligram of chemically pure indigotin, obtained from Kahlbaum, in 1,000 c.c. of chloroform. A portion of this is sealed in a test tube and kept in a dark place.

If the total amount of chloroform used for extraction is equal to $a$, and the amount of chloroform used to dilute the 2 c.c. to the color of the standard equals $x$, the total amount of chloroform necessary to dilute all of the chloroform used in extraction equals $a \times \frac{x}{2}$.

The total number of c.c. used in the extraction and in the dilution of the extraction mixture represents, therefore, a bulk containing 1 milligram of indigo. Bouma has suggested having a series of standard tubes containing from 1 to 20 milligrams of indigo to the liter, in this way avoiding the necessity of gradual dilution of the chloroform extract. In normal urines 5 to 10 c.c. are usually all that is required to extract the whole amount. But in cases of peritonitis and intestinal obstruction, Strauss has observed figures as high as 468.

To obtain the results in milligrams one must remember that the amount of indigo extracted was from 8 c.c. of urine, because the addition of the lead acetate solution diluted the specimen to that amount.

Drugs interfere but rarely with the color of the chloroform extract. Iodine can be removed by addition of a crystal of sodium thiosulphate to the colored extract after removal of the supernatant acid.

**Obermayer-Wang Method.**—Ellinger[1] has reviewed the quantitative methods of Obermayer,[1] Wang,[2] and Bouma,[3] and recommends certain alterations in the technique which conduce to greater accuracy.

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[1] Zeit. f. phys. Chemie, 1903, Bd. xxxviii, p. 178.

One hundred c.c. of the urine to be tested are slightly acidified, if necessary, with acetic acid, and then precipitated with one-tenth bulk of a solution of lead subacetate (Liquor plumbi subacetici, Ph. Ger.), and filtered through a dry filter, returning if necessary to obtain a clear solution. This precipitation removes the urinary pigments and also certain drugs; for example, salicylic acid. If the specific gravity of the urine is near or over 1.040 it should be diluted with an equal bulk of water.

An aliquot part of the measured filtrate is taken and mixed with an equal bulk of Obermayer's reagent or fuming hydrochloric acid, as preferred by Maillard.[4] The mixture is immediately shaken up with 30 c.c. of chloroform for two minutes. This process is repeated two or three times or until the chloroform is no longer colored blue. The chloroform extracts are united, washed with distilled water until no more acid is given off, filtered through dry paper into a carefully cleaned flask and heated over a water bath until all the chloroform has distilled off. The residue is then dried for five minutes on the bath, the mouth of the flask being left open. The dry residue is extracted three times with hot water, and if care is used the indigo will remain adherent to the glass. If particles do escape in the wash water, they should be collected on a filter, dried, and dissolved in chloroform.

Ten c.c. of pure, concentrated sulphuric acid are then added to the indigo residue and the mixture heated on a water bath until solution is effected. The acid must not decolorize a solution of potassium permanganate.

The indigo solution is now poured into an Erlenmeyer flask containing 100 c.c. of distilled water, and the washings of the first flask added. The blue solution should be clear, but a slight turbidity does not prevent an accurate determination. Permanganate solution is then run into the hot mixture from a burette until the reddish or greenish color is replaced by a pure yellow.

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[1] Zeit. f. phys. Chemie, 1903, Bd. xxxvi, p. 427.

[2] Zeit. f. phys. Chemie, 1898, Bd. xxv, p. 406; also, Zeit. f. phys. Chemie, Bd. xxvii, 1899, p. 135.

[3] Zeit. f. phys. Chemie, 1901, Bd. xxxii, p. 82.

[4] L'Indoxyle Urinaire, Paris, 1903.

The stock solution of standard permanganate is made by dissolving about 3 grams of the pure potassium salt[1] in 1,000 c.c. of distilled water. Five c.c. of this stock are diluted to 200 c.c. in a measuring flask, and this dilute mixture used for titration. The original stock solution must be standardized by titrating a weighed quantity of chemically pure indigo dissolved in 10 c.c. of sulphuric acid and diluted to about 100 c.c. The stock solution will keep for several months if protected from the light in a glass-stoppered bottle. A preliminary qualitative test should be made on the original urine to determine its indoxyl content; if this is high not more than 25 to 50 c.c. should be employed in the test; but 300 c.c. of normal urine may be used. The best results are obtained when from 10 to 15 c.c. of the permanganate are required for the decolorization. Bouma,[2] in a more recent paper, states that in his opinion the chloroform extract should be freed from acid by allowing it to run from the separatory funnel into distilled water. He also prefers to heat the residue for a half-hour on a water bath to remove impurities rather than to wash with hot water.

## AROMATIC OXYACIDS

These substances are derived from the tyrosin and phenylalanin group of the proteid molecule by putrefactive processes, and their excretion is usually parallel with that of the phenols. Among the acids of this type found in the urine are para-oxyphenyl-acetic and para-oxyphenyl-propionic acids. Oxymandelic and para-oxyhydro-cumaric acids are also occasionally met with in destructive diseases of the liver. They are excreted from the body in the form of salts.

The oxyacids have much the same diagnostic value as indoxyl.

**Qualitative Test.**—The urine is acidulated with hydrochloric acid and concentrated on a water bath to drive off the volatile phenols, cooled, and shaken out with ether. The combined extracts are shaken out with slightly alkaline water; the water acidulated and shaken out with ether; the ether evaporated; and the residue dissolved in distilled water and tested with

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[1] Kahlbaum's potassium permanganate is suitable for this purpose.

[2] Zeit. f. phys. Chemie, Bd. xxxix, 1903, p. 356.

Millon's reagent, which gives a red color. A precipitate is also formed on the addition of bromine water. No clinical interest is attached to the reactions for these acids.

### BENZOIC ACID

Traces of benzoic acid are occasionally found in the urine during the course of febrile diseases. It is produced by the splitting of hippuric acid by the action of the kidneys. The detection or estimation of this substance is of no clinical importance.

### OXYPROTEIC AND ALLOXYPROTEIC ACIDS

Oxyproteic acid, a substance which contains both nitrogen and sulphur, was discovered in the urine by Cloëtta.[1] Another body of similar structure, alloxyproteic acid, has been recently described.[2] Both acids are constant constituents of the urine and are derivatives of the albumin molecule. It has been thought that about 2 to 3 per cent. of the total nitrogen excretion is due to these substances. These acids do not react to the albumin tests, are precipitated by alcohol, but not by phosphotungstic acid or potassium ferrocyanide or acetic acid, nor do they give the biuret reaction; but they are precipitated by mercuric salts. The oxyproteic acid is distinguished from the alloxyproteic acid by its being precipitated with subacetate of lead and by the fact that it gives the diazo reaction. It is possible, therefore, that this acid is responsible for the Ehrlich reaction in typhoid fever. Some of the so-called neutral sulphur of the urine is no doubt contained in these acids. Their separation from the urine is a complicated process which need not be considered here.

### UROFERRIC ACID

Another acid of the sulphur-bearing group has been found in traces in normal urine by Thiele.[3] The substance seems closely related to the acids in the preceding paragraph and does not give the biuret reaction nor that of Millon or Adamkiewicz. It is precipitated by mercuric nitrate and sulphate and, in distinction to the acids mentioned above, forms an abundant precipitate with phosphotungstic acid.

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[1] Archiv f. exp. Path. u. Pharm., 1897, Bd. xl, p. 29.

[2] *St. Bondzynski* u. *K. Planck:* Chem. Berichte, 1902, Bd. xxxv, p. 2959.

[3] Diss., Leipzig, 1902.

### HIPPURIC ACID

This substance is contained in human urine, in amounts of from 1 to 3 decigrams. The quantity excreted is influenced considerably by the quantity and nature of the food taken in. For example, after the ingestion of 100 grams of grape sugar as high as 9 decigrams of hippuric acid have been found in the urine. During fever and intestinal putrefaction, and after the use of certain substances, such as quinic and salicylic acids, which are used as drugs, a considerable increase in the excretion of hippuric acid has been noted.

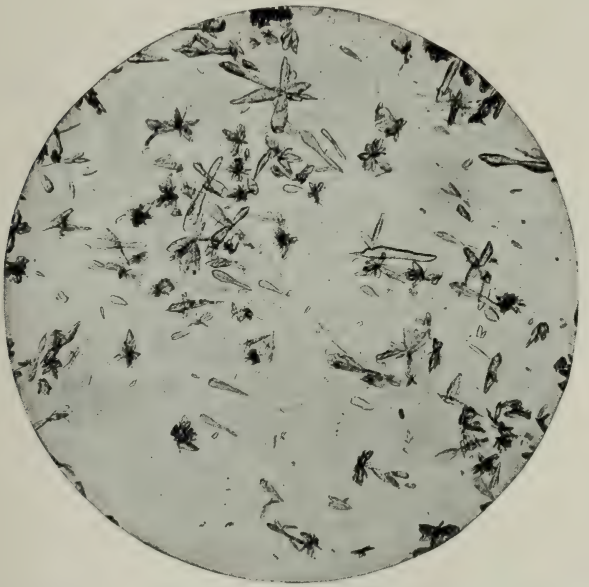

Fig. 134.—Crystals of Hippuric Acid from Urine. Magnified 150 diameters.

From a theoretical point of view hippuric acid is of interest, as it is one of the first substances whose synthesis in the organism has been thoroughly worked out. The process is a union of benzoic acid and glycocoll and takes place in the kidney and intestine. In the urine of the herbivorous animals hippuric acid is present in large amounts.

From a clinical point of view no facts of practical interest have as yet been obtained by the determination of the excretion of hippuric acid.

**The Quantitative Determination of Hippuric Acid.**—Bunge and Schmiedeberg[1] have devised a method for the determination of hippuric acid which gives excellent results in the urines containing large quantities of the acid. In human urine, the quantity of hippuric acid is usually too small to give good results.

Blumenthal[2] describes a method which is of use for the determination of small quantities. The criticism of this method by Soetbeer[3] has resulted in a slight correction by Blumenthal, which is said to give more accurate results.

Three hundred c.c. of the urine to be tested are rendered slightly alkaline with sodium carbonate solution and evaporated to dryness, first over a flame and then over a water bath. The residue is extracted twice, using each time 150 c.c. of 96 per cent. alcohol. The extraction should be carried out over a water bath, the resulting fluid filtered, and evaporated on a water bath to a syrup. The syrup is dissolved in 50 c.c. of water ; 10 c.c. of 25 per cent. hydrochloric or sulphuric acid is added and the mixture is shaken out in a separatory funnel with 200 c.c. of ether which contains 20 c.c. of 96 per cent. alcohol.

The shaking should be thorough and access of air to the funnel should be occasionally permitted. The ethereal extract is washed once with 75 c.c. of distilled water and the ether evaporated. This shaking out with ether is repeated four times, each extract to be washed with 75 c.c. of water. The ether residue is dissolved in 20 c.c. of distilled water and poured into a Kjeldahl flask. If the aqueous solution contains a large amount of coloring matter it should be shaken up with 15 c.c. of chloroform, before pouring into the flask. Fifteen c.c. of concentrated sulphuric acid are added to the contents of the flask, the mixture shaken, copper sulphate added, and the determination of the nitrogen carried out in the usual manner. The number of c.c. of decinormal sulphuric acid which are used is multiplied by 17.9, which gives the number of milligrams of hippuric acid in 300 c.c. of urine.

The results of this method are about 15 per cent. lower than those obtained by Bunge and Schmiedeberg ; but as the process is simpler and more quickly carried out it furnishes excellent comparative results when used in a series of determinations of the excretion of hippuric acid. If large quantities of the acid are excreted, the first mentioned process should be used.

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[1] Archiv f. exp. Path., Bd. vi, 1877, p. 235.

[2] Zeit. f. klin. Med., Bd. xl, 1900, p. 339.

[3] Beiträge z. chem. Phys. u. Path., 1902, vol. iii, p. 385. For a criticism of Blumenthal's method see *Hupfer :* Zeit. f. phys. Chem., Bd. xxxix, 1903, p. 302.

### ALKAPTONURIA

Urine is occasionally seen which turns black when exposed to the air, especially if rendered alkaline. Such urine also reduces Fehling's solution like a sugar, but does not ferment with yeast, and is optically inactive. Persons who have this peculiarity of metabolism are as a rule otherwise healthy, though some cases of ochronosis and progressive articular disease seem to have been closely related to alkaptonuria. The condition is extremely rare, is seen in men more often than in women, and shows a distinct tendency to occur in families.[1]

While the condition has been recognized for some two hundred years, Marshall[2] first isolated from the urine an acid substance which he designated glycosuric acid. This acid was later identified as homologous with gentisic acid, and was consequently called homogentisic acid.[3] For some time it was thought that another acid, uroleucinic,[4] was present, but recent investigations have not confirmed this opinion.

The substance is produced by the abnormal transformation in the body of p-tyrosin and phenylalanin, coupled with an inability to catabolize the homogentisic acid so formed.[5]

The amount of homogentisic acid excreted may be quite large, even 6 to 10 grams in twenty-four hours; but the quantity depends upon the amount of the above mentioned amino acids in the food.

The importance of recognizing the presence of these acids in the urine lies in the fact that persons suffering from this disorder are often refused by life insurance companies, being supposed to have diabetes because of the marked reducing action of the acids on Fehling's solution. Negative results, however, obtained with bismuth, fermentation, phenylhydrazin, orcin, and polariscopic tests, at once exclude any member of the sugar group. The excretion of these substances seems to have no effect on the health of the patient.

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[1] An excellent review of the subject with bibliography up to 1898 will be found in *Futcher:* New York Med. Jour., 1898, lxvii, p. 69. The admirable monograph of *Garrod* on "Inborn Errors of Metabolism" (London, 1909) contains an important chapter bringing the subject of alkaptonuria up to 1909.

[2] *Marshall:* Medical News, 1887, l, p. 35.

[3] *Kirk:* Jour. Anat. and Physiol., 1889, xxiii, p. 69.

[4] *Garrod* and *Hurtley:* Jour. Physiol., 1905, xxxiii, p. 206.

[5] *Dakin:* Jour. Biol. Chem., 1911, ix, p. 151.

**Qualitative Test.**—Garrod[1] recommends the following test:

The urine is heated to boiling and 5 or 6 grams of neutral lead acetate are added to each 100 c.c. A grayish precipitate is produced as soon as the salt dissolves and the mixture should be promptly filtered. The filtrate is placed in a cool spot for twenty-four hours, and if homogentisic acid is present the crystals of that substance, in combination with the lead, will separate out. The composition of the salt is $(C_8H_7O_4)_2$ Pb + $3H_2O$.

The acid may be set free from the lead salt by suspending 5 grams of the finely powdered substance in 50 c.c. of ether and passing a current of hydrogen sulphide. After filtering from the lead sulphide, the ether is evaporated and the pure acid separates. The crystals melt at 147° C.

### PYROCATECHIN AND HYDROCHINON

**Pyrocatechin** is found in traces in normal urine, more abundantly after the ingestion of phenol and benzol. Urine containing this substance darkens on standing, especially if alkaline.

**Hydrochinon** is present in the urine as an ethereal sulphate under the same conditions as pyrocatechin, and also causes blackening in alkaline urine.

**Tests.**[2]—The urine is strongly acidulated with hydrochloric acid, boiled for a short time, and after cooling, shaken out several times with ether. The ethereal extract is evaporated to a small bulk. The ether is shaken out with a watery sodium carbonate solution and the ether evaporated. The ethereal residue is dissolved in about 20 c.c. of a saturated solution of sodium chloride, filtered from the phenol and p-cresol, and the salt solution distilled after dilution with water, so long as the volatile phenols pass over. This is preferably carried on in a current of steam. The residue in the still is shaken out with ether, the ether evaporated, and the residue again distilled in water and precipitated with lead acetate, avoiding an excess. Pyrocatechin is precipitated; hydrochinon remains in the filtrate. The precipitate is mixed with water, decomposed with dilute sulphuric acid, and shaken out with ether. The pyrocatechin crystallizes from the ethereal solution and can be purified by recrystallization from hot benzol.

1. A watery solution gives a green color with dilute ferric chloride, which changes to violet on addition of a little tartaric acid and ammonia. 2. Fehling's solution is reduced.

The filtrate from the precipitate of the pyrocatechin is decomposed with sulphuric acid, shaken out with ether, and the ether evaporated. A brownish residue is recrystallized from boiling benzol.

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[1] Jour. of Phys., vol. xxiii, 1898–99, p. 512. *Orton* and *Garrod:* ibid., vol. xxvii, 1901, p. 89.

[2] *Baumann:* Zeit. f. phys. Chemie, Bd. viii, 1884, p. 188.

## MONO-AMINO ACIDS

### LEUCIN AND TYROSIN

These two substances are produced by the decomposition of the proteids, and are present in large amounts in the urine, chiefly in diseases of the liver, notably acute yellow atrophy, and to a less extent in phosphorus poisoning. They have been found in traces after the absorption of a pneumonic exudate when large quantities of the metabolic products of the proteids of the exudate are rapidly thrown into the circulation.

In a few cases of acute degeneration of the liver of a type differing from acute yellow atrophy, and also in phosphorus poisoning, acetone, diacetic acid, and a great increase in the ammonia excretion have been noted, leucin and tyrosin also being present in the urine. Occasionally glucose has also been found.[1]

Considerable amounts of leucin and tyrosin have also been found in a number of diseases, but not with sufficient frequency to warrant any diagnostic conclusions. Among these conditions may be mentioned erysipelas, typhoid fever, variola, rabies, leukæmia, gout, and diabetes.[2]

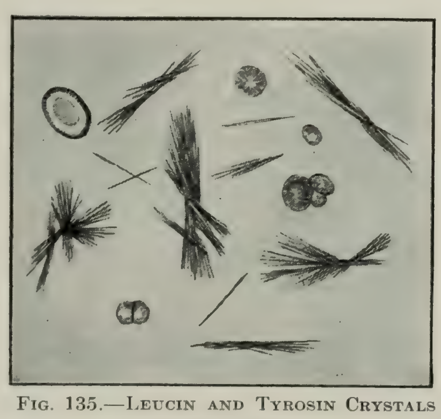

FIG. 135.—LEUCIN AND TYROSIN CRYSTALS IN THE URINE.

Tyrosin is found in the urine in the form of slender needles, and leucin in the form of highly refractile yellowish masses which have an oily look and a radial striation (Fig. 135). As a rule, they do not form spontaneous deposits except when present in the urine in large quantities, and it is usually necessary to evaporate the urine to obtain the crystals. It is easy to be misled by this procedure, for a normal urine frequently furnishes deceptive crystals when evaporated, so that if there is any reason for suspecting the presence of these two bodies they should be removed from the urine and tested in a pure state.

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[1] *Soetbeer:* Arch. f. exp. Path. u. Pharm., Bd. l, 1903, p. 295. *Naunyn:* Diabetes Mellitus, Wien, 1901, p. 48.

[2] *Ignatowski:* Zeit. f. phys. Chemie, Bd. xlii, 1904, p. 378.

**Isolation of Leucin and Tyrosin from the Urine.**—At least 500 c.c. of the urine, from which the albumin has been removed by heat, are precipitated with basic acetate of lead and the filtrate freed from a possible excess of lead by a stream of hydrogen sulphide. The filtrate from the lead sulphide is concentrated as much as possible and the residue treated with small quantities of absolute alcohol to remove the urea. Finally, the residue is treated with alcohol containing a little ammonia, and this ammoniacal solution is allowed to evaporate and is examined microscopically for the characteristic crystals. The leucin is more soluble in alcohol than the tyrosin, and if the mixture of the two is extracted with a small quantity of that substance the leucin will be extracted and leave a more or less pure tyrosin.

If a mixture of dry leucin and tyrosin is heated with a small amount of glacial acetic acid and an equal bulk of absolute alcohol, the leucin will be dissolved and the tyrosin remain as a white crystalline mass.[1]

The following chemical tests should be applied:

**Tests for Tyrosin.**—1. Gives Millon's reaction.

2. A hot solution when acidified with 1 per cent. acetic acid and then a little sodium nitrite added, turns bright red.

3. Tyrosin is heated with a few drops of sulphuric acid, the mixture diluted and neutralized with some barium carbonate. A violet color is produced on the addition of some neutral ferric chloride to the filtrate.

4. A small amount of the crystals to be tested is added to a few cubic centimeters of a reagent of the following composition:

1 c.c. formalin,
45 c.c. distilled water,
55 c.c. concentrated sulphuric acid.

The mixture is heated. If tyrosin is present a green color will be produced.[2]

**Tests for Leucin.**—1. Some leucin heated on platinum foil with a little nitric acid melts and forms an oily drop which rolls about on the platinum.

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[1] *Habermann* u. *Ehrenfeld:* Zeit. f. phys. Chemie, Bd. xxxvii, 1902, p. 18.

[2] *Morner:* Zeit. f. phys. Chemie, 1902, Bd. xxxiv, p. 207. *Denigès:* Comp. Rend., tome cxxx, 1900, p. 583.

2. Heated in a test tube leucin volatilizes in the form of white masses.

3. Leucin dissolves in strong NaOH, but not in ether.

4. Some copper sulphate and sodium hydrate are added to an aqueous solution of leucin, when the copper oxide is dissolved with the formation of a copper compound of leucin, and on heating the solution no reduction takes place.

## GLYCOCOLL

This substance is chemically amino-acetic acid. It is produced during the metabolism of the proteids, especially gelatin, and is also formed during the decomposition of uric acid, xanthin, and other purin bodies. It has been found in the urine in health, in experimental phosphorus poisoning,[1] gout, pneumonia and leukæmia,[2] and is interesting chiefly in connection with the appearance of the other members of the amino-acid group in the urine under similar conditions. The method of separation and identification of this substance as a $\beta$-naphthalin-sulpho-amino acid is too complicated for clinical work, and for further details the reader is referred to the original papers on the subject.

## CYSTIN

Cystin ($C_6H_{12}N_2O_4S_2$) belongs to the group of amino acids and is occasionally found in the urine as a result of a congenital anomaly of the metabolism of the proteids. It is occasionally accompanied in its excretion by certain diamins, notably cadaverin and putrescin.

The most generally accepted theory of the origin of cystin is that it results from the imperfect oxidation of the cystin radicle of the proteid of the food, but possibly may also be connected with excessive putrefaction of the proteid. The amount excreted per day may be a few centigrams or as high as one gram.

Cystinuria frequently appears in a number of members of the same family, as has also been noted to be the case with alkaptonuria.

An interesting observation illustrating the occurrence of cystinuria in members of the same family has been recently published by Abderhalden.[1] Attention was called to the disease by the discovery of numerous white particles scattered throughout the organs of a child dying of inanition at about the twenty-first month of its age. Examination of these white masses showed that they were composed of cystin, which could be isolated from the organs by extracting with ammonia and allowing the ammoniacal solution to evaporate spontaneously, the cystin crystallizing out in hexagonal plates. Two other children of the same family had died in infancy with the same symptoms. From the urine of the two surviving children cystin was isolated as a $\beta$-naphthalin-sulpho-compound.[2] The same compound was obtained from the urine of the father, but that of the mother was negative. A positive result, however, was obtained from the grandfather of the male line. That of the grandmother of the male line contained no cystin. In these cases no diamins could be demonstrated.

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[1] *Abderhalden* u. *Bergell:* Zeit. f. phys. Chemie, Bd. xxxix, 1903, p. 464.

[2] *Ignatowski:* Zeit. f. phys. Chemie, Bd. xlii, 1904, p. 378.

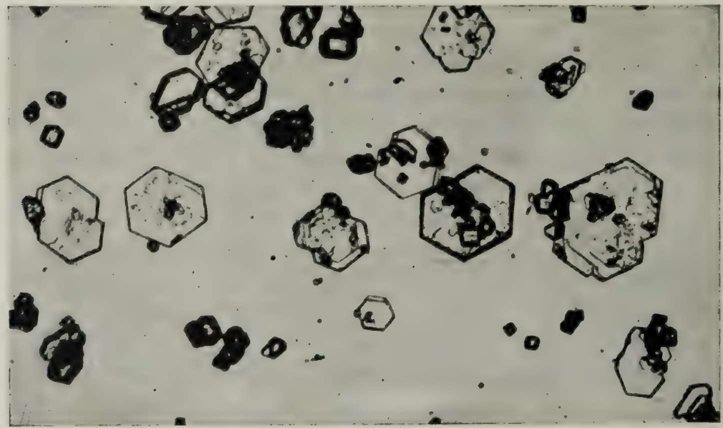

FIG. 136.—CRYSTALS OF CYSTIN FROM URINE.

The diagnosis of cystinuria is usually made by the discovery of calculi in the bladder, as the excretion of this substance is not connected with any general clinical symptoms.

**Qualitative Tests.**—Some strong acetic acid should be added to the urine and the fluid should be placed in a cool spot for twenty-four hours. At the end of that time the sediment from cystin urine will contain numerous flat, hexagonal plates (see Fig. 136). If the urine has already fermented the cystin may be deposited, as it is not soluble in ammonium carbonate. The crystals are easily soluble in ammonia and can be precipitated by the addition of acetic acid. The neutral sulphur of the urine is greatly increased in amount in cystinuria.

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[1] Zeit. f. phys. Chemie, Bd. xxxviii, 1903, p. 557.

[2] See *Fischer* and *Bergell:* Chem. Berichte, Bd. xxxv, 1902, p. 3784.

**Quantitative Tests.**—An approximately quantitative test[1] may be obtained by adding a considerable quantity of strong acetic acid to the urine, collecting the cystin which separates on a filter, drying, and weighing, and then making a separate determination of the neutral sulphur of the urine. An excessive amount of this substance may be attributed to cystin and added to the quantity obtained by direct weighing.

### DIAMINS (*Ptomaïns*)

Two substances belonging to the diamin group, cadaverin and putrescin, have been found in the urine and fæces of a few cases of cystinuria, but whether the two conditions are invariably associated is not as yet fully decided. The present belief is that diaminuria and cystinuria are both the expression of a marked impairment in the normal oxidation processes of the body.[2] The quantity of the diamins which may be found in the urine is variable. Simon was able to isolate 1.6 grams of the benzoyl compound from a twenty-four hours' specimen, but generally only traces have been found.

## CREATININ AND CREATIN

*Creatinin* ($C_4H_7N_3O$), an anhydride of creatin, is present in every normal urine. The quantity of creatinin excreted by a healthy person on a mixed diet is constant for each individual, and amounts to between 19 and 30 milligrams per kilo of body weight. Women excrete slightly smaller amounts than men; thin persons more in proportion than fat. Nursing infants excrete 4 to 11 milligrams per kilo; those fed on cow's milk slightly larger amounts. The ingestion of large quantities of meat or of meat extract increases both the creatin and the creatinin excretion, which probably accounts for the increase noted in diabetes. Muscular activity does not alter the excretion unless the subject is undernourished. In starvation the creatinin excretion sinks, being replaced by creatin. In fever the amount of creatinin is increased,[1] but the increase is not parallel to the total nitrogen excretion. In the insane with alternating periods of excitement and depression, the creatinin varies correspondingly. Low figures have been observed in diseases of the liver and in exophthalmic goitre. Creatinin is retained by the kidneys in advanced cases of nephritis, though it has nothing to do with the symptoms of uræmia.

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[1] *Simon:* Cystinuria and Its Relation to Diaminuria, Amer. Jour. Med. Sciences, 1900, vol. cxix, p. 39.

[2] *Moreigne:* Arch. de Méd. exper. et d'Anat. path., vol. xi, 1899, p. 254. *Simon:* Amer. Jour. Med. Sciences, 1900, vol. cxix, p. 39. *Baumann* u. *v. Udranszky:* Zeit. f. phys. Chemie, 1889, Bd. xiii, p. 362.

Both creatin and creatinin have a slight reducing power on hot Fehling's solution, but copper oxide is as a rule not separated. Creatinin is precipitated by phosphotungstic acid in the presence of hydrochloric acid, and also by solutions of zinc chloride and picric acid; creatin is not. The creatinin-zinc-chloride salt forms fine needles arranged in rosettes or balls.

**Qualitative Tests.**—The two important tests are as follows:

*Weyl's Reaction.*[2]—To the urine to be tested is added a very dilute watery solution of freshly dissolved sodium nitro-prusside, and then a few drops of dilute sodium hydrate. If creatinin be present the fluid becomes a brilliant cherry red and in a short time changes to yellow. Creatin does not give this reaction, but acetone does give a red color which changes on addition of acetic acid into violet. Acetone can be removed by a preliminary heating of the urine. The test indicates 6 parts in 10,000 of creatinin.

*Jaffé's Test*[3] is made by adding a small quantity of a saturated solution of picric acid to the urine to be tested and then a few drops of sodium hydrate. If creatinin is present a reddish color appears immediately which increases in intensity and remains unchanged for a long time. Acetone and diacetic acid give a weak yellowish red color, much less intense than the color produced by creatinin. The reaction indicates one part of creatinin in 5,000.

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[1] *Senator:* Virchow's Archiv, Bd. lxviii, 1876, p. 422.

[2] Chem. Berichte, vol. xi, 1878, p. 2175.

[3] Zeit. f. phys. Chemie, Bd. x, 1886, p. 399; also ibid., Bd. xiv, 1890, p. 471.

**Quantitative Tests.**—The old method is that of Neubauer and Salkowski,[1] in which the creatinin is precipitated as a zinc compound, but it has been largely replaced by the following simpler procedure:

*Folin's Method.*[2]—The reagents required are: 1, potassium bichromate solution containing 24.55 grams per liter; 2, saturated picric acid solution; 3, ten per cent. sodium hydrate solution.

In a 500 c.c. volumetric flask are placed 10 c.c. of the urine, 15 c.c. of the picric acid solution, and 5 c.c. of the sodium hydrate solution. The mixture is allowed to stand for five minutes, at the end of which time the flask is filled to the 500 c.c. mark with water. One of the cylinders of a Duboscq colorimeter is partly filled with this mixture, the other with the bichromate solution to the 8 mm. mark, and several readings are made immediately, the average being taken.

While the urine is standing it is well to adjust the eye by making a few preliminary readings, filling both cylinders with bichromate and setting one to the 8 mm. mark, and then trying to read as closely as possible with the other.

To calculate the amount of creatinin, multiply the number of c.c. of urine employed by the quotient obtained by dividing 8.1 by the number of millimeters of the urine solution required to give the same depth of color as 8 mm. of the bichromate. If the urine should be found to contain more than 15 or less than 5 mg. of creatinin to the 10 c.c. of urine taken the determination should be repeated with a correspondingly different amount of urine, as outside of these limits the method is less accurate.

*Creatin* ($C_4H_9N_3O_2$) is present in the urine of healthy persons when on a diet containing either meat or meat extracts. If the diet is composed wholly of starch, sugar, and fats, only traces appear; when on such a diet, one gram of creatin appears in the urine for each six introduced by mouth. The amount of creatinin under these circumstances is not altered. Creatin is found in the urine of undernourished and starving people in amounts of 0.15 to 0.5 gram. It is abundant in the urine of infants suffering from malnutrition. It is also found in considerable quantities in acute febrile conditions, diabetes, and exophthalmic goitre. Large quantities are excreted in some cases of carcinoma with rapid loss of weight. In the first few days post partum, quantities of 0.5 to 1.5 grams have been noted in twenty-four hours, probably due to involution of the uterine muscle. In diseases with muscular wasting, such as anterior poliomyelitis, the creatin excretion may be greatly increased; on the other hand, cases of marked muscular atrophy have been found to give normal figures.

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[1] Zeit. f. physiol. Chemie, Bd. xiv, 1890, p. 113.

[2] Ibid., xli, 1904, p. 223; also see Amer. Jour. of Physiol., xiii, 1905, p. 49,

Qualitative tests are of no interest.

**Quantitative Tests.**—There is no direct method for determining creatin in the urine. This substance must, therefore, be transformed into creatinin and estimated by the Folin method. In order to accomplish this, 20 c.c. of perfectly fresh urine are heated with 10 c.c. of normal hydrochloric acid for three hours on a water bath. The solution is then made up to 50 c.c. with distilled water and 25 c.c. of the fluid, corresponding to 10 c.c. of urine, are placed in a 500 c.c. flask with a small piece of litmus paper. About 10 c.c. of normal sodium hydrate are added, or until the fluid is approximately neutral. Fifteen c.c. of saturated picric acid solution and 5 c.c. of a 10 per cent. sodium hydrate solution are added; the mixture is allowed to stand for five minutes, and then diluted to 500 c.c.; and the total creatinin is determined as in the preceding paragraph. The creatin is found in terms of creatinin by subtracting the amount just obtained from the amount obtained by direct creatinin determination on the untreated urine. If it is desired to report the amount found in terms of creatin, the figures for creatinin should be multiplied by 1.16.

If an autoclave is available the 20 c.c. of urine can be mixed with an equal volume of normal hydrochloric acid, and this mixture heated for thirty minutes at a temperature of 117° to 120°, neutralized, and diluted to 50 c.c., and the determination carried out as above.[1] Urines containing large amounts of aromatic bodies often turn very dark on heating with acid. Such urines should be carefully neutralized after heating, precipitated with a small quantity of lead acetate, and the remaining lead removed either with sodium sulphate or with hydrogen sulphide. An aliquot part of the clear solution can then be used in the colorimeter after the addition of picric acid and sodium hydrate.

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[1] *Benedict* and *Meyer:* Amer. Jour. Physiol., 1907, xviii, p. 397.

## ALLANTOIN

This substance is a product of oxidation of uric acid and the autolysis of the liver, spleen, pancreas, etc.[1] It is found in human urine after the ingestion of large quantities of tannic acid, in the urine of new-born children, in the amniotic fluid, in traces in the urine of pregnant women, and in ascitic fluid and leukæmic blood. No clinical facts of importance are connected with the presence of this substance in the urine.

**Quantitative Test.**[2]—The urine to be tested is rendered faintly acid, and is precipitated with mercurous nitrate solution. The precipitate is filtered off, washed, and the filtrate decomposed by a current of hydrogen sulphide. The solution of mercurous nitrate should have a small amount of metallic quicksilver mixed with it. After filtering off the precipitate the filtrate is boiled and mixed with some magnesium oxide and silver nitrate solution is added to the mixture. The precipitate and the excess of magnesium oxide which it contains are oxidized by the Kjeldahl procedure and the amount of nitrogen determined. One c.c. of $^{N}/_{10}$ acid is equivalent to 0.0039 gram of allantoin.

## TOTAL NITROGEN

The determination of the total nitrogen of the urine has recently become of considerable interest from the evidence which it affords of the amount and rate of the excretion of the nitrogen derived from the proteid of the food, and especially so as the determination of urea as an evidence of disease has lost the position which it once held in the opinion of practitioners, and has become chiefly of historical interest.

The total nitrogen of the urine plus that of the fæces includes the whole of the nitrogenous excretion of the body, the excretion in the urine being from 92 to 93 per cent. of the total nitrogenous intake. The amount escaping from the lungs and skin can be neglected.

The amount of nitrogen excreted daily in a fasting condition is about 5.25 grams, corresponding to 33 grams of body proteid.

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[1] *Pohl :* Arch. f. exp. Path. u. Pharm., Bd. xlviii, 1902, p. 367; also *Mendel* and *Brown :* Am. Jour. of Phys., vol. iii, 1900, p. 261.

[2] *Loewi :* Arch. f. exp. Path. u. Pharm., Bd. xliv, 1900, p. 19 ; *Swain :* Amer Jour. of Phys., vol. vi, 1902, p. 38.

A normal adult on a mixed diet excretes from 11 to 15 grams of nitrogen per day, or 0.2 grams of nitrogen per kilo of body weight. If large amounts of nitrogenous food are consumed the excretion is apt to be considerably larger. A marked retention of nitrogen can be produced by an excessive proteid diet, so that for a considerable number of days 8 or 10 grams of nitrogen may be held in the body to be excreted later. A similar phenomenon has been observed after the administration of lecithin, which seems to produce a marked retention of nitrogen even when a person is on a diet containing normal quantities of proteids. The exact condition in which this nitrogen is stored up is not at present known, but it is probably not in the form of muscle proteid, because of the rapidity with which it is excreted when the organism contains a surplus. Probably the stimulus of the urea in the blood acting upon the kidneys starts up diuresis and the retained nitrogen is swept out of the body in the course of a few days.

The total nitrogen excretion of the urine is made up of a number of factors. About 86 per cent. of the nitrogen is due to urea; about 8 per cent. is derived from ammonia, purin bodies, creatinin, and pigments, of which the purin nitrogen forms about 2 per cent. and the ammoniacal nitrogen 2.5 per cent. The remainder, some 6 per cent., is made up of hippuric acid and undetermined bodies[1] of the amino group. The nature of the albumin contained in the food does not exert a very marked influence in the proportion of these substances, except on the amino-acid factor, the largest excretion of these substances being noted on a pure meat diet and the least on a milk diet.

An increase in the excretion of nitrogen, that is, a diminution of the nitrogen contained in the organism, is a physiological phenomenon in infants for three or four days after birth. It is also seen in all diseases accompanied by marked malnutrition or starvation, and is noted in the different types of fever due to sepsis or any other form of infection. The increased destruction of proteid under these circumstances depends upon a number of factors, one of which is the diminution in the carbohydrates and fat of the food which occurs during the fever owing to loss of appetite, etc.; another is the increase in temperature; but the most important factor is the breaking down of proteid due to the toxic substances produced by the bacteria. A similar toxic metabolism is especially well seen in persons the subjects of carcinoma or other malignant growths, and in pernicious anæmia. The same phenomenon is also seen in the chronic infectious diseases such as tuberculosis, in the leukæmias, scurvy, phosphorus poisoning, and exophthalmic goiter. The changes in the last condition are usually assumed to be due to circulating toxalbumin derived from the thyroid gland.

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[1] Further details on this subject will be found in papers by *v. Jaksch:* Zeit. f. klin. Med., Bd. xlvii, 1902, p. 1, and in Bd. l, 1903, p. 167.

An increase in the nitrogen excretion is occasionally seen in diabetes, but usually this is apparent only because of the great increase in the nitrogenous intake of these persons and from the fact that a diabetic can not "spare" his proteid by the use of carbohydrates or fats, so that the amount of these substances necessary for the body must be obtained by the splitting of the proteid either of the food or of the body. An increase in the nitrogen excretion is often noted in cases of nephritis with a high degree of albuminuria, the loss in this case being due to the nitrogen content of the albumin excreted. Large quantities of nitrogen are excreted during the resorption of an exudate.

A diminution in the output of nitrogen is chiefly noted in convalescent persons in whom a marked nitrogen retention is often seen, owing to the fact that the proteid of the food is being used to supply the albuminous needs of the body as the latter repairs the losses incurred during the illness.

In nephritis, also, especially in the later stages of the chronic diffuse forms of the disease, a considerable retention of nitrogen is not infrequent. In such conditions a marked increase in the excretion of nitrogen in the fæces can be observed, especially when the nephritis is accompanied by diarrhœa. The excretion through the skin is also greatly increased when retention reaches a high point. Even the mucous membrane of the stomach may act vicariously in place of the kidneys, and excrete considerable quantities of nitrogenous substances. The periods of retention in nephritis are varied by periods of excessive excretion, so that no diagnostic value can be attached to a single determination, especially if the nitrogenous content of the food taken in is not known.

The proper method of determining the retention of nitrogen in nephritis is to feed a patient for a number of days with measured quantities of milk, the nitrogen content of which has been determined, the nitrogen of the urine being also estimated each day.

If then the patient is given a meal very rich in proteid, a great increase in the urinary nitrogen will be noted if the kidneys are normal or occasionally if the kidneys are the site of a parenchymatous nephritis, while in some cases of chronic diffuse nephritis no immediate increase in the nitrogen excretion will be noted, but a gradual rise for several days until the surplus has been entirely removed. In not all cases is this phenomenon observed, and care must be taken not to confuse the diuresis and increased nitrogen excretion which sometimes takes place in a chronic nephritic owing to the stimulating action of the urea on the kidneys, for the rapid excretion of a normal kidney. The practitioner evidently gains but few facts of purely diagnostic nature from the estimation of the total nitrogen of the urine. The effects of diuresis and the simultaneous sweeping out of large quantities of nitrogen in the urine after suitable medication may, however, be best determined by this procedure, especially if the chlorides are also estimated. Some facts of practical importance in the prognosis and treatment of nephritis may therefore be obtained.

**Kjeldahl Method [1] for the Determination of the Total Nitrogen in the Urine.**—Ten c.c. of urine are carefully measured off into a round-bottomed flask of Jena glass and a few drops of a concentrated copper sulphate solution, 15 c.c. of sulphuric acid and 10 grams of potassium sulphate, are added. The flask should be supported in an inclined position so that none of the fluid can spurt out of the neck. The mouth of the flask should be loosely closed by a glass bulb blown on the end of a piece of glass tubing.

A convenient support for the flask is a thick piece of asbestos board with a hole in the center of sufficient size to permit the flame to come in contact only with that portion of the flask covered by the fluid, but not to permit of a direct flame touching the flask above the level of the fluid. A piece of wire gauze should protect the bottom of the flask from direct action of the flame.

The heating should be quite gentle for half an hour, but as soon as the foaming ceases the flame should be raised until the acid begins to boil gently. The whole heating process must be carried out under a hood in order to remove the sulphurous acid fumes which are given off. When the fluid in the flask is colorless or a pale green the oxidation process is completed. This requires at least an hour, though if very accurate results are required the heating should be from two to three hours, as creatinin, uric acid, and other substances are not easily oxidized.[1] The acid and the oxidizing agent ($CuSO_4$) change all of the nitrogenous bodies into ammonium sulphate. At the end of this time the liquid is cooled and prepared for distillation either in the same flask or washed out into another larger flask, which may be of Jena glass, but is preferably of copper, as the former is liable to break if the bumping is marked or heating is irregular. Two hundred c.c. of water are added and, after the fluid has cooled, enough of a 30 per cent. sodium hydrate solution to render the liquid strongly alkaline. The ammonia which is set free by the action of the alkali is distilled over into 80 c.c. of decinormal sulphuric acid, which has been accurately measured by a burette or a calibrated pipette.

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[1] Zeit. f. anal. Chemie, Bd. xxii, 1883, p. 378.

The flask is heated until about two-thirds of the fluid have passed over and there is considerable bumping from the separation of the sodium sulphate. This usually requires about thirty minutes. In order to prevent continuous bumping of the fluid it is well to add some fragments of pumice stone, a few pieces of granulated zinc, or some talc powder, at the beginning of the distillation.

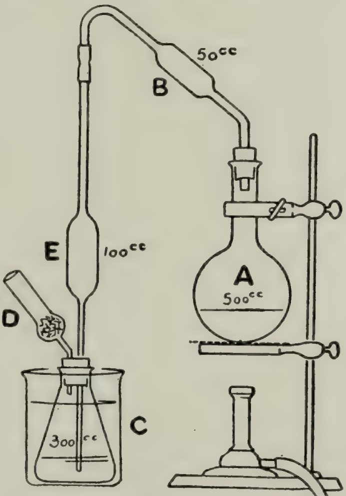

FIG. 137.—KJELDAHL APPARATUS. (Birch.)

A simple form of apparatus which can be extemporized from materials at hand in any laboratory is shown in Fig. 137. The tube *D* contains a few glass beads, and some of the sulphuric acid is poured over these to prevent the escape of any ammonia A few drops of methyl orange are added to the pearls and the flask *C* in order to indicate any change to alkalinity, in which case more acid should be promptly added. *D* is not necessary if a Liebig condenser is used. The tube *B* prevents the alkaline fluid in *A* from spurting over into *E*. The tubes *E* and *B* are made of broken pipettes of 50 or 100 c.c. capacity.

---

[1] *Kutscher* u. *Steudel:* Zeit. f. phys. Chemie, Bd. xxxix, 1903, p. 12. *Beger, Fingerling* u. *Morgan:* ibid., p. 329. *Malfatti:* ibid., p. 467. *Sörensen* u. *Pederson:* ibid., p. 513.

The decinormal acid into which the ammonia has condensed is titrated with decinormal sodium hydrate, using methyl orange as an indicator. The number of cubic centimeters of the $^{N}/_{10}$ NaOH used, subtracted from the amount of acid taken, gives the amount of ammonia which has distilled over—i.e., every cubic centimeter of acid neutralized by the ammonia is equivalent to so much decinormal ammonia. Decinormal ammonia contains 1.4 grams of nitrogen to the liter, or 0.0014 grams to 1 c.c., so that the amount of nitrogen can easily be computed by multiplying the number of c.c. of acid neutralized by 0.0014, which gives the nitrogen in grams in 10 c.c. of urine. This method gives a very accurate determination of the total nitrogen excreted in the urine, including that from the urea, uric acid, creatinin, ammonia, and amino acids.

## UREA

Urea is $CON_2H_4$ or $CO\begin{smallmatrix}\diagup NH_2\\ \diagdown NH_2\end{smallmatrix}$. It is formed chiefly in the liver, but probably also in other organs, from the end products of proteid digestion, presumably the amino groups. The exact steps of the synthesis between the amino bodies on the one side, and urea on the other, are not known.

In the normal human urine, urea is found in amounts of from 20 to 30 grams in 24 hours. An approximate estimation in proteid-free urine can be obtained by multiplying the total nitrogen in grams by 2. Women secrete less than men. Children absolutely less, but relatively more when the body weight is taken into consideration.

The amount excreted in health is dependent very largely on the quantity of proteid in the diet. In those who are on a milk diet and in poorly fed working people whose nitrogen intake is low because of the relatively small quantity of meat consumed, the amount may be as little as 15 grams a day. On a mixed diet, about 85 per cent. of the nitrogen excreted is in the form of urea; on a strictly vegetable diet, 79 per cent.; and on a diet rich in proteid, 87 per cent.

The curve of excretion is irregular, with two elevations, one shortly after a meal, due to the absorption of the products of gastric proteolysis, and the other four to seven hours after eating, due to the absorption of the products of intestinal digestion.

In health, urea is not all excreted promptly, but in irregular periods; usually a slight retention occurs lasting for a few days and then the surplus is rapidly excreted. A slight increase is seen after drinking large quantities of water, after severe exercise, and after certain drugs, such as caffeine, theobromine, quinine, salicylic acid, etc.

In disease, an increased secretion is seen in fever, when the destruction of the proteids is increased; and in diabetes, when the same conditions apply as in the total nitrogen excretion. A marked increase in the excretion is seen during the resorption of an exudate or the resolution of a pneumonia.

A marked diminution in the excretion of urea is frequently present in diseases of the liver, especially those accompanied by extensive destruction of the glandular substance. Under these conditions the total excretion of nitrogen may be approximately normal or only slightly diminished, but the urea excretion falls considerably, and its place is taken by an increase in the amount of the amino acids, the chief of which are leucin and tyrosin. In some cases of acute degeneration of the liver the ammonia excretion reaches a high point and the urea is diminished without any increase in the amino acids.

A diminished amount of urea is also found in starvation, though the decrease is somewhat marked in that due to an obstructive carcinoma of the stomach or œsophagus, by the increase in nitrogenous metabolism which results from the toxic effects of the new growth.

In acute nephritis there may or may not be a diminution in the excretion of urea due to retention in the body. In chronic parenchymatous nephritis in the early stages the urea excretion may be normal, but often shows fluctuations somewhat greater than those observed in persons with healthy kidneys. In the later stages of the disease when the diagnosis can be made without the assistance of chemical analyses, the urea is often greatly diminished in amount and the urea content of the blood increased.

In some cases of nephritis where a marked retention exists the urea seems to exert some influence on the production of œdema, for if these patients are given five or ten grams of urea by mouth marked diuresis will occasionally be produced with the sweeping out of the surplus urea and the reduction of the œdema. Other cases are on record, however, in which the administration of comparatively small doses of urea has determined an extensive œdema and a fatal termination of the disease.

The retention of urea in obscure cases of chronic diffuse nephritis is unfortunately neither of sufficient degree nor constancy to warrant the drawing of important diagnostic or prognostic conclusions therefrom.

The practitioner would do well to spend his time examining the urine for casts and albumin rather than in making quantitative determinations of urea. In the early cases where the diagnosis is important from the point of view of prognosis and treatment, urea may be excreted in a perfectly normal manner. In the later cases the diagnosis of nephritis can be made more simply and surely by a physical examination of the patient and a study of the albumin reactions and a microscopical examination of the urinary sediment.

### QUALITATIVE TESTS FOR UREA

The urea crystallizes in needles or long, colorless, rhombic prisms. The melting point of these crystals is 130° C. At room temperatures urea is soluble in an equal weight of water and in 5 parts of alcohol, the latter property being frequently made use of in extracting urea from urinary residues obtained by concentrating urine by evaporation. Urea is insoluble in pure ether or chloroform.

Urea nitrate is formed on the addition of nitric acid to urea. Its crystalline form is that of thin, rhombic or hexagonal, colorless plates, the most acute angle being 82°. The morphology of these crystals is frequently of importance in demonstrating small quantities of urea in cyst fluids. The fluid is concentrated and mixed with a small quantity of strong nitric acid. The nitrates of the alkalies, however, form crystals which resemble urea nitrate, and a portion of the crystalline product should always be tested by heating for the formation of biuret.

**Biuret Reaction.**—Urea when heated gives off ammonia and leaves an opaque white residue which contains cyanuric acid and biuret. The residue is dissolved in water and treated with an excess of alkali and a trace of copper sulphate, when a beautiful reddish violet color will be produced which is the biuret reaction.

**Schiff's Reaction.**—Two drops of a concentrated furfurol solution, four to six drops of twenty per cent. hydrochloric acid, and a small crystal of urea are mixed. In a few minutes a deep violet color appears in the fluid.

Another test which may be made use of depends upon the reaction with sodium hypobromite in which urea is broken up with the formation of sodium bromide, carbon dioxide, and nitrogen. The formula is given in the next paragraph.

### QUANTITATIVE ESTIMATION OF UREA

**Hypobromite Method.**—The procedure most frequently used to determine the urea in the urine is that dependent upon the decomposition of that substance with sodium hypobromite and the measurement of the gas evolved.

The reaction is as follows: $CON_2H_4 + 3NaOBr = 3NaBr + 2N + CO_2 + 2H_2O$. As will be noted from the formula, the carbonic acid is set free in a gaseous form, but practically the solution is so arranged that the $CO_2$ is absorbed by an excess of alkali always present.

The solution of hypobromite is prepared by taking 70 c.c. of a 30 per cent. stock solution of sodium hydrate and diluting it with 180 c.c. of water, and then adding 5 c.c. of bromine and stirring until all the bromine has been dissolved. The solution thus made up will keep for about a week if kept in a cool, dark place. The bromine should be mixed with the sodium hydrate solution only after the latter has cooled, and the whole process should be carried on under a hood or out of doors. If this is not possible, some aqua ammonia should be poured about the desk near the beaker in which the mixture is being made, to neutralize the fumes of the bromine and to prevent their irritating action on the mucous membranes of the nose of the operator.

Another method, which does not require the use of a stock solution of sodium hydrate, is to make up a solution of 200 grams of NaOH in 500 c.c. of water and then to add 12.5 c.c. of bromine after the mixture has cooled.

The urine must be free from sugar and albumin and should contain less than 1 per cent. of urea. If the urine contains more as it usually does, a rapid preliminary estimation will enable one to dilute it to the proper strength.

In order to measure the gas evolved some form of graduated apparatus is necessary, and for the practitioner the most convenient procedure is to have the graduations so arranged that the readings can be made directly in grams to the c.c. The most convenient apparatus of this type is the Doremus ureometer, and preferably the more recent modification with a side tube to admit the urine, which obviates the use of a pipette. The instrument, as made by the glass-blower, is easily upset, so that it is well to embed the glass foot in a cake of plaster of Paris some three inches in diameter, in order to render the apparatus more steady.

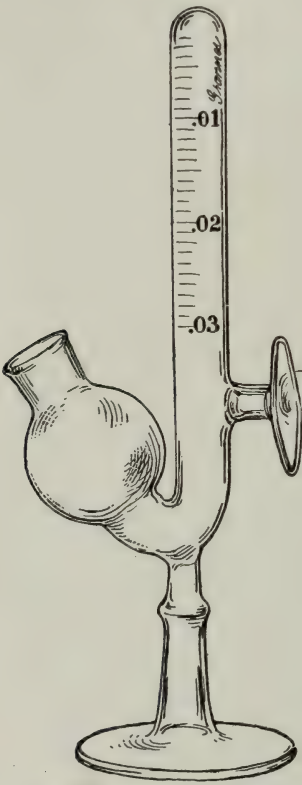

Fig. 138.—Doremus Ureometer. Improved form.

The closed portion of the U-tube is filled with the hypobromite solution, and the urine introduced either with the pipette or by allowing it to run in from the side tube by opening the glass cock arranged for that purpose. After all the gas has risen and the instrument has stood for a short time the readings may be made in grams to the liter, or in percentage. The results of the most careful determinations with this apparatus are extremely inaccurate, so there is no need of correction for temperature and barometric pressure.

Much more satisfactory results may be obtained by the use of a Lunge gas burette arranged as shown in Fig. 139, in which larger quantities of urine can be decomposed by the hypobromite and more accurate determinations made. The graduated tube and the adjusting tube are filled with either water, glycerin, or old hypobromite solution, the last being preferable.

The small bottle at the side is filled with about 25 c.c. of hypobromite solution. Two c.c. or more of urine are measured off and placed in a small test tube which is carefully inserted in the bottle so that none of the contents mix with the hypobromite. The cork is then pushed tightly into the neck of the bottle. The Lunge stop-cock is so arranged that the apparatus communicates with the air, in order to equalize the pressure, and the column of water is brought to the top of the graduated tube by raising the other tube. The cock is then turned so that the bottle containing the urine and hypobromite is in communication with the graduated tube. The bottle is then inclined so that the urine mixes with the hypobromite solution, the nitrogen is evolved, and the fluid falls in the graduated tube. The carbon dioxide is promptly absorbed by the alkali, and in about half an hour the pressure of the inclosed nitrogen is equalized by lowering the side tube until the fluid is the same level in both.

The volume of gas liberated is then read off and if 2 c.c. of urine has been used, the amount of urea is obtained directly from the table, using that one whose temperature corresponds most closely to the temperature of the room. It is scarcely necessary to correct for atmospheric pressure and the tension of the water vapor, as the errors due to these factors are within the general error of the method.

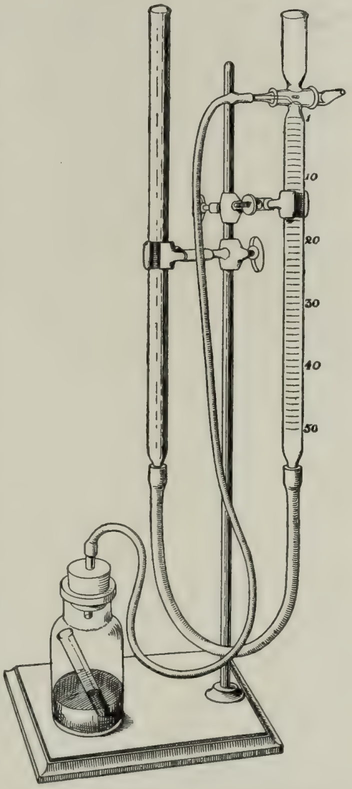

Fig. 139.—Ureometer with Lunge Burette.

The following tables from Simon and Regnard allow direct reading of the quantity of urea corresponding to a given amount of nitrogen at temperatures of 15°, 20°, 25°, and 30° C.

## UREA.—TABLE FOR A TEMPERATURE OF 15° C.

| | 1 | $\frac{1}{10}$ | $\frac{2}{10}$ | $\frac{3}{10}$ | $\frac{4}{10}$ | $\frac{5}{10}$ | $\frac{6}{10}$ | $\frac{7}{10}$ | $\frac{8}{10}$ | $\frac{9}{10}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| 1..... | 1.28 | 1.41 | 1.53 | 1.66 | 1.79 | 1.92 | 2.04 | 2.17 | 2.30 | 2.43 |
| 2..... | 2.56 | 2.69 | 2.81 | 2.94 | 3.07 | 3.20 | 3.33 | 3.46 | 3.58 | 3.71 |
| 3..... | 3.84 | 3.97 | 4.10 | 4.22 | 4.35 | 4.48 | 4.61 | 4.74 | 4.87 | 4.99 |
| 4..... | 5.12 | 5.25 | 5.38 | 5.50 | 5.63 | 5.76 | 5.89 | 6.02 | 6.14 | 6.27 |
| 5..... | 6.40 | 6.53 | 6.60 | 6.79 | 6.91 | 7.04 | 7.17 | 7.30 | 7.43 | 7.55 |
| 6..... | 7.68 | 7.81 | 7.94 | 8.07 | 8.19 | 8.32 | 8.45 | 8.58 | 8.71 | 8.83 |
| 7..... | 8.96 | 9.09 | 9.22 | 9.35 | 9.48 | 9.60 | 9.73 | 9.86 | 9.99 | 10.12 |
| 8..... | 10.24 | 10.37 | 10.50 | 10.63 | 10.76 | 10.88 | 11.01 | 11.14 | 11.27 | 11.40 |
| 9..... | 11.53 | 11.65 | 11.78 | 11.91 | 12.04 | 12.17 | 12.29 | 12.42 | 12.55 | 12.68 |
| 10..... | 12.81 | 12.93 | 13.06 | 13.19 | 13.32 | 13.45 | 13.57 | 13.70 | 13.83 | 13.96 |
| 11..... | 14.09 | 14.22 | 14.34 | 14.47 | 14.60 | 14.73 | 14.86 | 14.98 | 15.11 | 15.24 |
| 12..... | 15.37 | 15.50 | 15.62 | 15.75 | 15.88 | 16.01 | 16.14 | 16.26 | 16.39 | 16.52 |
| 13..... | 16.65 | 16.78 | 16.91 | 17.03 | 17.16 | 17.29 | 17.42 | 17.55 | 17.67 | 17.80 |
| 14..... | 17.93 | 18.06 | 18.19 | 18.31 | 18.44 | 18.57 | 18.70 | 18.83 | 18.95 | 19.08 |
| 15..... | 19.21 | 19.34 | 19.47 | 19.60 | 19.72 | 19.85 | 19.98 | 20.11 | 20.24 | 20.36 |
| 16..... | 20.49 | 20.62 | 20.75 | 20.88 | 21.00 | 21.13 | 21.26 | 21.39 | 21.52 | 21.64 |
| 17..... | 21.77 | 21.90 | 22.03 | 22.16 | 22.29 | 22.41 | 22.54 | 22.67 | 22.80 | 22.93 |
| 18..... | 23.05 | 23.18 | 23.31 | 23.44 | 23.57 | 23.69 | 23.82 | 23.95 | 24.08 | 24.21 |
| 19..... | 24.34 | 24.46 | 24.59 | 24.72 | 24.85 | 24.98 | 25.10 | 25.23 | 25.36 | 25.49 |
| 20..... | 25.62 | 25.74 | 25.87 | 26.00 | 26.13 | 26.26 | 26.38 | 26.51 | 26.64 | 26.77 |
| 21..... | 26.90 | 27.03 | 27.15 | 27.28 | 27.41 | 27.54 | 27.67 | 27.79 | 27.92 | 28.05 |
| 22..... | 28.18 | 28.31 | 28.43 | 28.56 | 28.69 | 28.82 | 28.95 | 29.07 | 29.20 | 29.33 |
| 23..... | 29.46 | 29.59 | 29.72 | 29.84 | 29.97 | 30.10 | 30.23 | 30.36 | 30.48 | 30.61 |
| 24..... | 30.74 | 30.87 | 31.00 | 31.12 | 31.25 | 31.38 | 31.51 | 31.64 | 31.76 | 31.89 |
| 25..... | 32.02 | 32.15 | 32.28 | 32.41 | 32.53 | 32.66 | 32.79 | 32.92 | 33.05 | 33.17 |
| 26..... | 33.30 | 33.43 | 33.56 | 33.69 | 33.81 | 33.94 | 34.07 | 34.20 | 34.33 | 34.45 |
| 27..... | 34.58 | 34.71 | 34.84 | 34.97 | 35.10 | 35.42 | 35.35 | 35.48 | 35.61 | 35.74 |
| 28..... | 35.86 | 35.99 | 36.12 | 36.25 | 36.38 | 36.50 | 36.63 | 36.79 | 36.89 | 37.02 |
| 29..... | 37.15 | 37.27 | 37.40 | 37.53 | 37.66 | 37.79 | 37.91 | 38.04 | 38.17 | 38.30 |
| 30..... | 38.43 | 38.55 | 38.68 | 38.81 | 38.94 | 39.07 | 39.12 | 39.32 | 39.45 | 39.58 |

## UREA.—TABLE FOR A TEMPERATURE OF 20° C.

| | 1 | $\frac{1}{10}$ | $\frac{2}{10}$ | $\frac{3}{10}$ | $\frac{4}{10}$ | $\frac{5}{10}$ | $\frac{6}{10}$ | $\frac{7}{10}$ | $\frac{8}{10}$ | $\frac{9}{10}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| 1..... | 1.26 | 1.38 | 1.51 | 1.63 | 1.76 | 1.89 | 2.01 | 2.14 | 2.26 | 2.39 |
| 2..... | 2.52 | 2.64 | 2.77 | 2.90 | 3.02 | 3.16 | 3.27 | 3.40 | 3.53 | 3.65 |
| 3..... | 3.78 | 3.91 | 4.03 | 4.16 | 4.28 | 4.41 | 4.54 | 4.66 | 4.79 | 4.91 |
| 4..... | 5.04 | 5.17 | 5.29 | 5.42 | 5.54 | 5.67 | 5.80 | 5.92 | 6.05 | 6.17 |
| 5..... | 6.30 | 6.43 | 6.55 | 6.68 | 6.81 | 6.93 | 7.06 | 7.18 | 7.31 | 7.44 |
| 6..... | 7.56 | 7.69 | 7.81 | 7.94 | 8.07 | 8.19 | 8.32 | 8.44 | 8.57 | 8.70 |
| 7..... | 8.82 | 8.95 | 9.08 | 9.20 | 9.33 | 9.45 | 9.58 | 9.71 | 9.83 | 9.96 |
| 8..... | 10.08 | 10.21 | 10.34 | 10.46 | 10.59 | 10.71 | 10.84 | 10.97 | 11.09 | 11.22 |
| 9..... | 11.35 | 11.47 | 11.60 | 11.72 | 11.85 | 11.98 | 12.10 | 12.23 | 12.35 | 12.48 |
| 10..... | 12.61 | 12.73 | 12.86 | 12.98 | 13.11 | 13.24 | 13.36 | 13.49 | 13.61 | 13.74 |
| 11..... | 13.87 | 13.99 | 14.12 | 14.25 | 14.37 | 14.50 | 14.62 | 14.75 | 14.88 | 15.00 |
| 12..... | 15.13 | 15.25 | 15.38 | 15.51 | 15.63 | 15.76 | 15.88 | 16.01 | 16.14 | 16.26 |
| 13..... | 16.39 | 16.52 | 16.64 | 16.77 | 16.89 | 17.02 | 17.15 | 17.27 | 17.40 | 17.52 |
| 14..... | 17.65 | 17.78 | 17.90 | 18.03 | 18.15 | 18.28 | 18.41 | 18.53 | 18.66 | 18.78 |
| 15..... | 18.91 | 19.04 | 19.16 | 19.29 | 19.42 | 19.54 | 19.67 | 19.79 | 19.92 | 20.05 |
| 16..... | 20.17 | 20.30 | 20.42 | 20.55 | 20.68 | 20.80 | 20.93 | 21.05 | 21.18 | 21.31 |
| 17..... | 21.43 | 21.56 | 21.69 | 21.81 | 21.94 | 22.06 | 22.19 | 22.32 | 22.44 | 22.57 |
| 18..... | 22.69 | 22.82 | 22.95 | 23.07 | 23.20 | 23.32 | 23.45 | 23.53 | 23.70 | 23.83 |
| 19..... | 23.96 | 24.08 | 24.21 | 24.33 | 24.46 | 24.59 | 24.71 | 24.84 | 24.96 | 25.09 |
| 20..... | 25.22 | 25.34 | 25.47 | 25.59 | 25.72 | 25.85 | 25.97 | 26.10 | 26.22 | 26.35 |
| 21..... | 26.48 | 26.60 | 26.73 | 26.86 | 26.98 | 27.11 | 27.23 | 27.36 | 27.49 | 27.61 |
| 22..... | 27.74 | 27.86 | 27.99 | 28.12 | 28.24 | 28.37 | 28.49 | 28.62 | 28.75 | 28.87 |
| 23..... | 29.00 | 29.13 | 29.25 | 29.38 | 29.50 | 29.63 | 29.76 | 29.88 | 30.01 | 30.13 |
| 24..... | 30.26 | 30.39 | 30.51 | 30.64 | 30.76 | 30.89 | 31.02 | 31.14 | 31.27 | 31.39 |
| 25..... | 31.52 | 31.65 | 31.77 | 31.90 | 32.03 | 32.15 | 32.28 | 32.40 | 32.53 | 32.66 |
| 26..... | 32.78 | 32.91 | 33.03 | 33.16 | 33.29 | 33.41 | 33.54 | 33.66 | 33.79 | 33.92 |
| 27..... | 34.04 | 34.17 | 34.30 | 34.42 | 34.55 | 34.67 | 34.80 | 34.93 | 35.05 | 35.18 |
| 28..... | 35.30 | 35.43 | 35.56 | 35.68 | 35.81 | 35.93 | 36.06 | 36.19 | 36.31 | 36.44 |
| 29..... | 36.57 | 36.69 | 36.82 | 36.94 | 37.07 | 37.20 | 37.32 | 37.45 | 37.57 | 37.70 |
| 30..... | 37.83 | 37.95 | 38.08 | 38.20 | 38.33 | 38.46 | 38.58 | 38.71 | 38.83 | 38.96 |

## UREA.—TABLE FOR A TEMPERATURE OF 25° C.

| | 1 | $\frac{1}{10}$ | $\frac{2}{10}$ | $\frac{3}{10}$ | $\frac{4}{10}$ | $\frac{5}{10}$ | $\frac{6}{10}$ | $\frac{7}{10}$ | $\frac{8}{10}$ | $\frac{9}{10}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| 1..... | 1.24 | 1.36 | 1.49 | 1.61 | 1.73 | 1.86 | 1.98 | 2.11 | 2.23 | 2.35 |
| 2 .... | 2.48 | 2.60 | 2.73 | 2.85 | 2.97 | 3.10 | 3.22 | 3.35 | 3.47 | 3.59 |
| 3..... | 3.72 | 3.84 | 3.97 | 4.09 | 4.22 | 4.34 | 4.46 | 4.59 | 4.71 | 4.84 |
| 4..... | 4.96 | 5.08 | 5.21 | 5.33 | 5.46 | 5.58 | 5.70 | 5.83 | 5.95 | 6.08 |
| 5..... | 6.20 | 6.33 | 6.45 | 6.57 | 6.70 | 6.82 | 6.95 | 7.07 | 7.19 | 7.32 |
| 6..... | 7.44 | 7.57 | 7.69 | 7.81 | 7.94 | 8.06 | 8.19 | 8.31 | 8.43 | 8.50 |
| 7 .... | 8.68 | 8.81 | 8.93 | 9.06 | 9.18 | 9.30 | 9.43 | 9.55 | 9.68 | 9.80 |
| 8..... | 9.92 | 10.05 | 10.17 | 10.30 | 10.42 | 10.54 | 10.67 | 10.79 | 10.92 | 11.04 |
| 9..... | 11.17 | 11.29 | 11.41 | 11.54 | 11.66 | 11.79 | 11.91 | 12.03 | 12.16 | 12.28 |
| 10..... | 12.41 | 12.53 | 12.65 | 12.78 | 12.90 | 13.03 | 13.15 | 13.27 | 13.40 | 13.52 |
| 11..... | 13.65 | 13.77 | 13.89 | 14.02 | 14.14 | 14.27 | 14.39 | 14.52 | 14.64 | 14.76 |
| 12..... | 14.89 | 15.01 | 15.14 | 15.26 | 15.38 | 15.51 | 15.63 | 15.76 | 15.88 | 16.00 |
| 13..... | 16.13 | 16.25 | 16.38 | 16.50 | 16.63 | 16.75 | 16.87 | 17.00 | 17.12 | 17.26 |
| 14..... | 17.37 | 17.49 | 17.62 | 17.74 | 17.87 | 17.99 | 18.11 | 18.24 | 18.36 | 18.49 |
| 15..... | 18.61 | 18.74 | 18.86 | 18.98 | 19.11 | 19.23 | 19.36 | 19.48 | 19.60 | 19.73 |
| 16..... | 19.85 | 19.98 | 20.10 | 20.22 | 20.35 | 20.47 | 20.60 | 20.72 | 20.84 | 20.97 |
| 17..... | 21.09 | 21.22 | 21.34 | 21.47 | 21.59 | 21.71 | 21.84 | 21.96 | 22.09 | 22.21 |
| 18..... | 22.33 | 22.46 | 22.58 | 22.71 | 22.83 | 22.95 | 23.08 | 23.20 | 23.33 | 23.45 |
| 19..... | 23.58 | 23.70 | 23.82 | 23.95 | 24.07 | 24.20 | 24.32 | 24.44 | 24.57 | 24.69 |
| 20..... | 24.82 | 24.94 | 25.06 | 25.19 | 25.31 | 25.44 | 25.56 | 25.68 | 25.81 | 25.93 |
| 21..... | 26.06 | 26.18 | 26.30 | 26.43 | 26.55 | 26.68 | 26.80 | 26.92 | 27.05 | 27.17 |
| 22..... | 27.30 | 27.42 | 27.55 | 27.67 | 27.79 | 27.92 | 28.04 | 28.17 | 28.29 | 28.41 |
| 23..... | 28.54 | 28.66 | 28.79 | 28.91 | 29.04 | 29.16 | 29.28 | 29.41 | 29.53 | 29.66 |
| 24..... | 29.78 | 29.90 | 30.03 | 30.15 | 30.28 | 30.40 | 30.52 | 30.65 | 30.77 | 30.90 |
| 25..... | 31.02 | 31.15 | 31.27 | 31.39 | 31.52 | 31.64 | 31.77 | 31.89 | 32.01 | 32.14 |
| 26..... | 32.26 | 32.39 | 32.51 | 32.63 | 32.76 | 32.88 | 33.01 | 33.13 | 33.25 | 33.38 |
| 27..... | 33.50 | 33.63 | 33.75 | 33.88 | 34.00 | 34.12 | 34.25 | 34.37 | 34.50 | 34.62 |
| 28..... | 34.74 | 34.87 | 34.99 | 35.12 | 35.24 | 35.36 | 35.49 | 35.61 | 35.74 | 35.86 |
| 29..... | 35.99 | 36.11 | 36.23 | 36.36 | 36.48 | 36.61 | 36.73 | 36.85 | 36.98 | 37.10 |
| 30..... | 37.23 | 37.35 | 37.47 | 37.60 | 37.72 | 37.85 | 37.97 | 38.09 | 38.22 | 38.34 |

## UREA.—TABLE FOR A TEMPERATURE OF 30° C.

| | 1 | $\frac{1}{10}$ | $\frac{2}{10}$ | $\frac{3}{10}$ | $\frac{4}{10}$ | $\frac{5}{10}$ | $\frac{6}{10}$ | $\frac{7}{10}$ | $\frac{8}{10}$ | $\frac{9}{10}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| 1..... | 1.22 | 1.34 | 1.46 | 1.58 | 1.71 | 1.83 | 1.95 | 2.07 | 2.19 | 2.32 |
| 2..... | 2.44 | 2.56 | 2.68 | 2.80 | 2.93 | 3.05 | 3.17 | 3.29 | 3.41 | 3.54 |
| 3..... | 3.66 | 3.78 | 3.90 | 4.03 | 4.15 | 4.77 | 4.39 | 4.51 | 4.64 | 4.76 |
| 4..... | 4.88 | 5.00 | 5.12 | 5.25 | 5.37 | 5.49 | 5.61 | 5.73 | 5.86 | 5.98 |
| 5..... | 6.10 | 6.22 | 6.35 | 6.47 | 6.59 | 6.71 | 6.83 | 6.96 | 7.08 | 7.20 |
| 6..... | 7.32 | 7.44 | 7.57 | 7.69 | 7.81 | 7.93 | 8.05 | 8.18 | 8.30 | 8.42 |
| 7..... | 8.54 | 8.67 | 8.79 | 8.91 | 9.03 | 9.15 | 9.28 | 9.40 | 9.52 | 9.64 |
| 8..... | 9.76 | 9.89 | 10.01 | 10.13 | 10.25 | 10.37 | 10.50 | 10.62 | 10.74 | 10.86 |
| 9..... | 10.99 | 11.11 | 11.23 | 11.35 | 11.47 | 11.60 | 11.72 | 11.84 | 11.96 | 12.08 |
| 10..... | 12.21 | 12.33 | 12.45 | 12.57 | 12.69 | 12.82 | 12.94 | 13.06 | 13.18 | 13.30 |
| 11..... | 13.43 | 13.55 | 13.67 | 13.79 | 13.92 | 14.04 | 14.16 | 14.28 | 14.40 | 14.53 |
| 12..... | 14.65 | 14.77 | 14.89 | 15.01 | 15.14 | 15.26 | 15.38 | 15.50 | 15.62 | 15.75 |
| 13..... | 15.87 | 15.99 | 16.11 | 16.24 | 16.36 | 16.48 | 16.60 | 16.72 | 16.85 | 16.97 |
| 14..... | 17.09 | 17.21 | 17.33 | 17.46 | 17.58 | 17.70 | 17.82 | 17.94 | 18.07 | 18.19 |
| 15..... | 18.31 | 18.43 | 18.56 | 18.68 | 18.80 | 18.92 | 19.04 | 19.17 | 19.29 | 19.41 |
| 16..... | 19.53 | 19.65 | 19.78 | 19.90 | 20.02 | 20.14 | 20.26 | 20.39 | 20.51 | 20.63 |
| 17..... | 20.75 | 20.88 | 21.00 | 21.12 | 21.24 | 21.36 | 21.49 | 21.61 | 21.73 | 21.85 |
| 18..... | 21.97 | 22.10 | 22.22 | 22.34 | 22.46 | 22.58 | 22.71 | 22.83 | 22.95 | 23.07 |
| 19..... | 23.19 | 23.32 | 23.44 | 23.56 | 23.68 | 23.81 | 23.93 | 24.05 | 24.17 | 24.29 |
| 20..... | 24.42 | 24.54 | 24.66 | 24.78 | 24.90 | 25.03 | 25.15 | 25.27 | 25.39 | 25.51 |
| 21..... | 25.65 | 25.76 | 25.88 | 26.00 | 26.13 | 26.25 | 26.37 | 26.49 | 26.61 | 26.74 |
| 22..... | 26.86 | 26.98 | 27.10 | 27.22 | 27.35 | 27.47 | 27.59 | 27.71 | 27.83 | 27.96 |
| 23..... | 28.08 | 28.20 | 28.32 | 28.45 | 28.57 | 28.69 | 28.81 | 28.93 | 29.06 | 29.18 |
| 24..... | 29.30 | 29.42 | 29.54 | 29.67 | 29.79 | 29.91 | 30.03 | 30.15 | 30.28 | 30.40 |
| 25..... | 30.52 | 30.64 | 30.77 | 30.89 | 31.01 | 31.13 | 31.25 | 31.38 | 31.50 | 31.62 |
| 26..... | 31.74 | 31.86 | 31.99 | 32.11 | 32.23 | 32.35 | 32.47 | 32.60 | 32.72 | 32.84 |
| 27..... | 32.96 | 33.09 | 33.21 | 33.33 | 33.45 | 33.57 | 33.70 | 33.82 | 33.94 | 34.06 |
| 28..... | 34.18 | 34.31 | 34.43 | 34.55 | 34.67 | 34.79 | 34.92 | 35.04 | 35.16 | 35.28 |
| 29..... | 35.41 | 35.53 | 35.65 | 35.77 | 35.89 | 36.02 | 36.14 | 36.26 | 36.38 | 36.50 |
| 30..... | 36.63 | 36.75 | 36.87 | 36.99 | 37.11 | 37.24 | 37.36 | 37.48 | 37.60 | 37.72 |

**Mörner-Sjoqvist Method.**[1]—This method is considered to be one of the most accurate. Five c.c. of the urine to be examined, from which albumin should be removed, are mixed with 5 c.c. of baryta mixture and 100 c.c. of alcohol and ether containing two volumes of 97 per cent. alcohol and one volume of anhydrous ether. The baryta mixture is composed of a saturated barium chloride solution containing 5 per cent. of barium hydrate. The combination is allowed to stand until the next day, is filtered, and the residue washed with fresh alcohol and ether mixture, and the combined filtrates evaporated at a temperature not exceeding 60° C. When the volume has been reduced to about 25 c.c., some water and a little calcined magnesia, stirred up in twelve parts of water, are added, and the whole heated to drive off the ammonia. The process is continued until the vapor does not show any alkalinity when tested with a moistened strip of litmus paper. The fluid and the residue are washed into a Kjeldahl flask of Jena glass, a little sulphuric acid is added, and finally, 10 c.c. of the concentrated acid; the mixture is heated, and the nitrogen determined as in the Kjeldahl process. One part of nitrogen is equivalent to 2.143 parts of urea.

In urines which contain large quantities of hippuric acid the method does not give correct results.[2] Braunstein[3] has therefore suggested a modification which gives a more accurate determination. After the evaporation of the ammonia as above the fluid is poured into a small flask containing ten grams of crystalline phosphoric acid, or the equivalent amount of solution, and the whole heated in a hot air sterilizer for four and one-half hours at a temperature between 140° and 145° C. The air bath should have a thermoregulator, so that the temperature is kept within these limits. After cooling, the residue is dissolved in water, the solution transferred to a Kjeldahl flask, rendered alkaline by the addition of 10 c.c. of 30 per cent. sodium hydrate, and the ammonia distilled off into decinormal sulphuric acid.

**Folin's Method.**[4]—Three c.c of the urine is measured into an Erlenmeyer flask of 200 c.c. capacity, and 2 c.c. of concentrated

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[1] Skand. Arch. f. Phys., Bd. ii, 1891, p. 438; also, Zeit. f. phys. Chemie, Bd. xvii, p. 140.

[2] *Salaskin* u. *Zaleski:* Zeit. f. phys. Chemie, Bd. xxviii, p. 73.

[3] Zeit. f. phys. Chemie, Bd. xxxi, 1901, p. 381.

[4] Zeit. f. phys. Chemie, Bd. xxxii, 1901, p. 504; ibid., Bd. xxxvii, p. 548.

HCl (sp. gr. 1.14) and twenty grams of magnesium chloride added. The flask is closed by a tight-fitting cork perforated for a vertical condensing tube of about 10 mm. diameter and 20 cm. length, to prevent the escape of all the hydrochloric acid. The contents of the flask are now boiled for ten minutes in order to remove the excess of water. At the end of this time the drops of condensed water, falling back into the hot mixture below, give rise to a sharp hissing sound. When this is noticed the flame should be lowered and the contents of the flask gently boiled for half an hour. Some distilled water is carefully allowed to run down the glass tube into the flask in order to prevent any separation of the magnesium chloride crystals, and then the solution is washed out into a liter flask and diluted to 500 c.c. Eight c.c. of 20 per cent. solution of sodium hydrate and a small amount of chalk are added, and the ammonia distilled off into a measured quantity of $^{N}/_{10}$ sulphuric acid, and the amount of free acid remaining is determined by titration with $^{N}/_{10}$ sodium hydrate as in the Kjeldahl process. The amount of distillate required to carry over all the ammonia is about 350 c.c. About one hour is sufficient to obtain this quantity. The distillate must be boiled before titration to remove any $CO_2$ which may be present.

Commercial magnesium chloride contains ammonia, so that the salt used must always be previously tested by the Kjeldahl method to determine the amount present, and this figure is to be subtracted from the result. Mörner[1] has carefully tested the method and finds that it gives excellent results, especially if the urine is first mixed with barium hydroxide. He suggests 5 c.c. of urine, 2 grams of barium hydroxide, and 100 c.c. of alcohol-ether mixture (one third ether). The urea is determined in the residue obtained after the evaporation of the alcohol-ether with some calcined magnesia. This modification is especially important when sugar is present.

**Schöndorff's Method.**[2]—The solutions required are as follows:

*a.* One hundred grams of phosphotungstic acid and 100 c.c. of hydrochloric acid of a specific gravity of 1.124 are placed in a measuring flask and the mixture filled up to 1,000 c.c. The cloudy solution is thoroughly shaken and filtered through asbestos fiber. If the cloud reappears the solution should be again filtered.

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[1] Skand. Arch. f. Phys., Bd. xiv, 1903, p. 297.

[2] Arch. f. d. Gesammte Phys., Bd. lxii, 1896, p. 1. The method here given is that recommended by *von Jaksch* and differs slightly from the original procedure as published by *Schöndorff*.

*b.* Ten per cent. solution of crystallized orthophosphoric acid in distilled water.

Twenty c.c. of the urine are measured out and precipitated with 60 c.c. of the phosphotungstic acid solution and allowed to stand for twenty-four hours.

Five c.c. of the same specimen of urine are precipitated with 15 c.c. of the same solution and filtered. To the filtrate are added several c.c. of the phosphotungstic acid. If a cloud appears in a few minutes an additional 5 c.c. of phosphotungstic acid should be added to the mixture of 20 c.c. of urine and 60 c.c. of phosphotungstic acid solution which was first made. This procedure should be repeated until further addition gives no precipitate, but an excess should be carefully avoided. Usually 60 c.c. of the phosphotungstic acid solution are sufficient for 20 c.c. of the urine. After the mixture of urine and phosphotungstic acid has stood for twenty-four hours it is filtered and the filtrate rubbed up with calcium hydrate until an alkaline reaction appears, which is made evident by the blue color of the mixture. The solution is then filtered through a Schleicher and Schüll No. 581 paper, and an aliquot part, usually 20 c.c. corresponding to 5 c.c. of the urine, is measured off. To this are added 100 c.c. of the phosphoric acid solution and the fluid is poured into a Kjeldahl flask of about 500 c.c. capacity. The flask is placed in a sand bath and surrounded by an asbestos hood with an opening above for the neck. The fluid in the flask is then heated to 150° C. for four hours, the temperature being observed by means of a thermometer. The residue is dissolved in water, 80 c.c. of a 25 per cent. sodium hydrate solution are added, and the whole is distilled after the addition of talc powder. The ammonia which passes over is collected in tenth normal sulphuric acid. One c.c. of decinormal sodium hydrate corresponds to 0.0014 gram of nitrogen. In order to compute the amount of urea this figure should be multiplied by 2.143.

The method can not be applied unless sugar is removed from the urine, and it is also necessary to test the reagents by a blank experiment in order to determine the amount of ammonia which may be present.

## URIC ACID

Uric acid is formed in the body by the decomposition of the nucleins of the nuclei of the cells of the food and of the body. These nucleins are of a very complicated structure and break up when digested into a series of compounds which are called collectively, the alloxur, xanthin, or purin bodies. The latter name is given because all these bases and uric acid seem to be derived from a hypothetical substance called by Fischer, purin.

Under perfectly normal conditions the twenty-four hour urine of an adult contains the widely varying amounts of from 0.39 to 2 grams of uric acid. In males the average of a large series of observations can be considered as from 0.5 to 0.75 gram ; in females 0.3 to 0.5 gram in twenty-four hours. The variations in the figures depend somewhat upon the accuracy of the different methods used in quantitative determinations, the difference in the food ingested, and to a considerable degree on the age and personal equation of the subject examined.

In infancy and especially during the first few days after birth, the excretion is relatively much higher than in later life. The variations due to personal idiosyncrasy are not dependent on the varying amounts of food used by the different persons, but upon some as yet little understood difference in individual metabolism.[1]

It has been found that a certain amount of the uric acid excreted is derived from the nucleins of the food, but if a person is put upon a nuclein-free food, such as milk, or fasts for a time, there is still a constant excretion of uric acid derived from the nuclei of the cells of the body. This excretion has been designated the endogenous purin, while that which is derived from the food has been termed exogenous. The latter is very variable and can be raised and lowered by a suitable diet. The endogenous purin, on the other hand, is quite constant, but varies in different individuals. It may be considered as from 0.4 to 0.57 gram per day.[2]

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[1] For the theories of the formation of uric acid, see *Wiener:* Asher-Spiro, Ergebnisse d. Physiologie, Bd. i, 1902, p. 555; also, *Halliburton:* Brit. Med. Journal, September, 1900, p. 735.

[2] *Burian* u. *Schur:* Pflügers Arch., Bd. lxxx, 1900, p. 241, and Bd. lxxxvii, 1901, p. 239. *Kaufmann* u. *Mohr:* Deut. Arch. f. klin. Med., Bd. lxxiv, 1902, p. 141.

The influence of food not containing an excess of nucleins upon the excretion of uric acid is slight. For instance, Hermann gives the following figures: Vegetable diet, 0.478 gram; mixed diet, 0.636 to 0.674 gram; meat diet, 0.981 gram. Meat causes an increase in the uric acid excretion because of its large content of extractives; pure proteid or albumose causes no increase, while Liebig's beef extract causes a large increase. Eggs and milk exert practically no increase as a rule; sometimes they decrease the output. Fats cause little or no change.

Tissues rich in nuclein, like thymus [1] and liver, cause almost constantly a considerable increase in the excretion of uric acid and may even double it; the same is seen after the ingestion of nuclein preparations. The effect of sugars or starch is not constant. The administration of large quantities may cause a transient increase, but only by their action on metabolism, not because they are capable of being transformed into uric acid. Drinking of large quantities of water increases the excretion slightly. Alcohol increases the amount excreted very slightly.

It is possible for the uric acid to be derived from the nuclei of the leucocytes, and an increase in the uric acid excretion is observed when there is a great destruction of these cells, such as occurs, for instance, in leukæmia, in which disease very large amounts of uric acid are excreted, rising even to 8 grams in twenty-four hours.

During a leucocytosis with only a moderate increase in the number of leucocytes the uric acid may or may not be perceptibly increased. This may be due to the fact that the increase of the leucocytes in the circulation may not be accompanied by increased destruction, and that the amount of uric acid excreted from the body does not furnish any clue to the amount formed.

In resolving pneumonia and acute degeneration of the liver, in both of which conditions there is great destruction of a tissue rich in nucleins, the amount of uric acid excreted may be very large.

Uric acid is also eliminated in the fæces in moderate amounts, so that the whole excretion can not be estimated by the analysis of the urine alone.

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[1] *Weintraud:* Berl. klin. Woch., 1895, p. 405. See also *Vogt:* Deut. Arch. f. klin. Med., Bd. lxxi, 1901, p. 21. *Reach:* Münch. med. Woch., 1902, p. 1215. (Both observers noted that an attack of gout followed ingestion of considerable amounts of sweetbread.)

Gout[1] has long been considered to be due to the increase of uric acid in the blood, and there are reasons for thinking that there may be such an increase in certain cases; but modern investigations with more accurate methods than those formerly employed have tended to cast some doubt upon the constancy of this condition, and it has been found that while in acute gout the amount of uric acid may be high, it is also often low and never in so large a quantity in the blood and urine as in leukæmia. Evidently the mere presence of an excess of uric acid does not determine an attack of gout.

It has been suggested that though in gout there may be no great increase in the amount of uric acid in the urine, yet the deposits of urates in the tissues in gout might be accounted for by the fact that the uric acid is formed locally in the tissues, and that the uric acid so formed incites the toxic symptoms.

It has also been suggested that the other purin bodies, xanthin, hypoxanthin, etc., are the toxic agents in gout.

The whole question is still unsettled and in need of fresh observations with accurate methods. Our present knowledge merely points to an underlying disorder of metabolism, the nature of which is not understood, but which is accompanied by deposition of urates in the tissues and a variation in the excretion of uric acid from the normal.

The ratio between the total nitrogen and the uric acid, and the ratio between the latter and urea, have been found so variable even in health that no facts of clinical importance can be derived from them. The same is true of the mere quantitative determination of the uric acid excretion, especially if the patient is on a mixed diet, for a meal containing moderate amounts of sweetbread, liver, or other food rich in nucleins, will raise the excretion of uric acid more than an attack of gout.

The quantitative estimation of the purin bodies has as yet produced no facts of clinical value. The diagnosis of gout must rest for the present upon clinical symptoms, and not upon chemical analyses of the urine.

Uric acid is of clinical interest from another point of view—

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[1] A very complete monograph with a full discussion of the theories concerning gout is that by *Minkowski:* Die Gicht, Nothnagel's Handbuch, Bd. vii, Wien, 1903; see also *Schreiber:* Ueber die Harnsäure, Stuttgart, 1899; and *Futcher:* Jour. Amer. Med. Ass'n, 1902, vol. xxxix, p. 1046.

that of the formation of uric-acid infarcts in the kidneys of the new-born and calculi in the urinary tract. For the identification of the uric acid in these situations the following tests are of value.

### QUALITATIVE TESTS FOR URIC ACID

*a.* A few crystals of the material are placed on a slide and dissolved in dilute NaOH. If now a drop of dilute HCl is allowed to flow under the cover glass, typical whetstone-shaped crystals will form of uric acid.

*b.* Murexide test. A small quantity of the specimen is moistened with $HNO_3$ in a small porcelain dish and gently warmed until a yellowish red residue is left; this is then treated with a very little ammonia, when the color will be altered to a beautiful purple color changing to a deep blue on the addition of a little NaOH. If the fluid is warmed the color disappears, and when evaporated the color does not reappear, which is a point of difference between uric acid and xanthin or guanin, the color returning in the case of the two purin bases mentioned.

*c.* Uric acid dissolved in dilute sodium carbonate and dropped on a paper wet with silver nitrate, leaves a dark spot of reduced silver.

*d.* Uric acid dissolved in dilute NaOH and treated with Fehling's solution gives a precipitate of white cuprous oxide in combination with uric acid, and finally of red cuprous oxide, this property of uric acid accounting for a portion of the normal reducing power of the urine. The reduction due to uric acid may be somewhat diminished in amount if the urine to be tested is filtered through animal charcoal before the titration.

There is no simple method of estimating the uric acid in the urine; the most accurate method is the Ludwig Salkowski, which should always be employed for scientific purposes.

### QUANTITATIVE DETERMINATION OF URIC ACID

**Ludwig-Salkowski Method.**[1]—The principles underlying this method are the precipitation of uric acid as a silver magnesium urate in the presence of magnesium salts, by ammoniacal silver solution, and the solubility of silver chloride in ammonium hydrate. Two hundred c.c. of urine, diluted so that its specific gravity does not exceed 1.020, are mixed with 50 c.c. of magnesia mixture in a graduated cylinder; made up with water to 300 c.c., and immediately filtered through a dry filter paper into a beaker. Two hundred c.c of the filtrate are measured off and 10 to 15 c.c. of a 3 per cent. solution of silver nitrate are added The precipitate must be of a flocculent, gelatinous nature If it is white, it is an evidence that too much chloride of silver is present, and some ammonia must be added and the whole thoroughly stirred. A few white granules of chloride of silver in the precipitate do not interfere with the accuracy of the method. The precipitate is allowed to settle and a small quantity of the supernatant fluid is removed with a pipette and acidulated with nitric acid. It should cloud from the formation of silver chloride, an evidence that an excess of silver is present in the solution. If this clouding does not appear, the sample should be rendered alkaline with ammonia, poured back into the main mass of the fluid and several c.c. of silver nitrate solution added. Occasionally it is necessary to add more ammonia. Then the supernatant fluid should be again tested by acidulation with nitric acid in the same manner as before. If an excess of silver is present the precipitate is filtered through a Schleicher and Schüll paper, No. 597, any particles adhering to the inside of the beaker carefully removed with a glass rod covered with soft rubber tubing, and washed on to the filter so that none of the precipitate is lost. The residue on the filter is then washed with water until the filtrate shows no cloudiness when acidulated with nitric acid; in other words, until no silver is present, and also until when on the addition of a little silver nitrate there is only a slight cloudiness, showing that only a small amount of chlorides are present. The funnel is now placed in the neck of a 500 c.c. flask. The tip of the filter is perforated with a glass rod and the precipitate carefully washed into the flask and shaken up. It is acidulated with a couple of drops of hydrochloric acid. Hydrogen sulphide is then passed through the mixture. The whole is frequently shaken until the filtrate is saturated with the gas. When this occurs the mixture is heated to the boiling point, filtered, and the flask washed out with hot water two or three times. It is important to examine the sulphide of silver which is retained on the filter in order to see whether any crystals of uric acid are present. The filtrate must be clear and colorless. If it is dark it must be immediately returned through the filter until the filtrate is perfectly clear. If only a little of the silver sulphide passes through it can be neglected.

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[1] *Salkowski:* Practicum, second edition, 1900.

The filtrate is evaporated, first over a flame, then over a water bath, until it is reduced to a few c.c. Five to eight drops of hydrochloric acid are added and the whole allowed to remain until the next day. The uric acid separates out in crystalline form and is usually only slightly colored. If any silver sulphide separates out during the process of evaporation it can be removed by filtration, but it is important not to filter after the volume of the solution is reduced to one-half, as otherwise some uric acid may be retained on the paper.

It is now necessary to determine the quantity of the uric acid which has separated out. For this purpose a nine centimeter filter is dried in a weighing bottle at 110° C., and weighed. The uric acid is then very carefully brought on to this filter paper, using a portion of the filtrate for the purpose of washing the crystals out of the beaker. As soon as they are completely transferred to the filter, the crystals are washed with a small quantity of water until the filtrate shows no reaction for chlorides. The filtrate and the wash water are collected and measured. The bulk should not exceed 50 or 60 c.c. The funnel is then filled twice with strong alcohol and once with ether, and the filter and its contents are dried and weighed. The difference between the original weight of the paper and the second result is the amount of uric acid. When small quantities of uric acid are present a correction should be made for the solubility of the uric acid in the filtrate plus the wash water. For each 10 c.c. of these fluids, five-tenths of a milligram of uric acid should be added to the total amount.

**Hopkins' Method as Modified by Folin.**[1]—The reagents required are—

*a.* A solution of 500 grams of ammonium sulphate, 5 grams of uranium acetate, and 60 c.c. of a 10 per cent. solution of acetic acid, dissolved in 650 c.c. of water.

*b.* A twentieth normal solution of potassium permanganate made by dissolving 1.581 grams of Kahlbaum's chemically pure potassium permanganate in 1,000 c.c. of water. If especially accurate results are desired the strength of the potassium permanganate should be determined by titration against a standard solution of oxalic acid, as given in the Appendix.

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[1] Zeit. f. phys. Chem., Bd. xxxii, 1901, p. 552.

*c*. Pure concentrated sulphuric acid. Three hundred c.c. of the urine to be tested are placed in a half liter flask and 75 c.c. of the ammonium sulphate mixture added in order to precipitate a mucoid substance which interferes with the precipitation of the ammonium urate.

After the mixture has stood for about five minutes it is filtered through a double folded filter and two portions each of 125 c.c. are measured off from the filtrate into two beakers. Five c.c. of concentrated ammonia are added to the contents of the beakers, and after stirring they are set aside until the next day. The supernatant fluid is carefully filtered through a Schleicher and Schüll filter, No. 597. The precipitated ammonium urate is washed on to the filter by means of a 10 per cent. ammonium sulphate solution, and then the filter is filled up twice with the same reagent. The titration is not interfered with by traces of chlorides, and the whole filtration and washing can be completed in from twenty to thirty minutes.

The urate precipitate is then washed into a beaker with about 100 c.c. of water; 15 c.c. of concentrated sulphuric acid are added and the solution immediately titrated while still warm. Toward the end of the reaction the permanganate solution should be added in portions of two drops each until a faint rose color is seen throughout the entire fluid. Each c.c. of twentieth normal permanganate solution corresponds to 3.75 milligrams of uric acid. Three milligrams of uric acid should be added to the results for each 100 c.c. of the urine used, on account of the solubility of ammonium urate which remains in the fluid and is not precipitated.

E. E. Smith[1] prefers to decompose the ammonium urate on the filter by means of hydrochloric acid and weigh the separated uric acid as in the Salkowski method.

Folin also suggests a modification of the Salkowski process by titrating the uric acid which separates instead of weighing it. The crystals of the acid should be dissolved by means of a small amount of chemically pure sodium hydrate solution. The hydrate used should not be the so-called "purified by alcohol," because this contains some sodium alcoholate which reduces permanganate, but the "pure from sodium." The solution should be made up to 100 c.c., the 15 c.c. of sulphuric acid added and the mixture titrated as above.

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[1] Oral communication.

### PURIN BODIES

The members of the purin group which have been found in the urine are xanthin, hypoxanthin, adenin, paraxanthin, heteroxanthin, episarkin, epiguanin, and methylxanthin.

The amount of these substances which is excreted in the urine in twenty-four hours is very small, and varies a good deal in different individuals. Unfortunately the analytical methods for the quantitative determinations of these substances are not above criticism and the results must be regarded as only approximate. It has been estimated that from 15 to 45 milligrams, chiefly of the xanthins, are excreted in the urine in twenty-four hours. These bodies appear to be chiefly derived from the theobromine, caffeine, and theophyllin contained in the food, the caffeine and theobromine being especially abundant in coffee, tea, and cocoa. There seems to be no doubt, however, that a portion of the purin bases are derived from the nucleins of the body and are therefore entitled to be considered as endogenous purins.

There is no simple method for the accurate quantitative determination of these substances, though Hall[1] has devised an apparatus for the estimation of the total purin excretion which furnishes approximate results. The method is based upon the measurement of the bulk of the precipitate of silver purin produced in a urine containing an excess of ammonia. It is evident that the results can be only approximate.

### THE DETERMINATION OF URIC ACID AND THE PURIN BASES IN THE URINE

**The Method of Krüger and Schmid.**[2]—This may be used to determine both the purin bases and uric acid. The principle of the process is to precipitate both the uric acid and the purin bases in combination with oxide of copper and then to decompose the precipitate with sodium sulphide. Hydrochloric acid will then separate the uric acid from the solution, while the purin bases pass through and can be separated from the filtrate by the formation of either copper or silver compound. Finally, the nitrogen of both the uric acid and the purin precipitate is determined by the Kjeldahl method.

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[1] Wien. klin. Woch., vol. xvi, 1903, p. 411.

[2] *Hoppe-Seyler:* Handbuch der chem. Analyse, p. 435.

The reagents necessary are:

1. Commercial sodium bisulphite solution (Kahlbaum).
2. Ten per cent. copper sulphate solution.
3. Sodium sulphide solution made up by saturating a 1 per cent. sodium hydrate solution with $H_2S$ and adding an equal bulk of 1 per cent. sodium hydrate.
4. A suspension of manganese dioxide in water, which may be obtained by heating a 0.5 per cent. solution of potassium permanganate with a little alcohol until it is decolorized.
5. Ten per cent. hydrochloric acid.
6. Ten per cent. acetic acid.
7. Solid sodium acetate.

Four hundred c.c. of the urine should be taken, the total excretion in twenty-four hours having been diluted so as to make this amount one-fourth or one-fifth of the total. Albumin if present is to be removed by heating with dilute acetic acid and filtering. The urine is then placed in a liter flask, 24 grams of sodium acetate and 40 c.c. of sodium bisulphite solution are added, and the whole heated to boiling, and, after the addition of 40 to 80 c.c. of the copper sulphate solution, is kept at the boiling point for at least three minutes. The amount of copper sulphate added depends upon the content of the specimen in purin bases. The flocculent precipitate which forms is filtered off, washed with hot water until the wash water is colorless, and then returned to the flask by puncturing the tip of the filter paper and washing through with hot water.

The addition of water is continued until the fluid amounts to about 200 c.c. It is then heated to boiling, and the copper oxide precipitate is decomposed by the addition of 30 c.c. of sodium sulphide solution. In general this amount is sufficient, but it is always advisable to see that there is an excess of sulphide present by placing a drop of the solution in contact with a drop of lead acetate, when a brown color will show an excess of sodium sulphide. As soon as decomposition is complete the mixture is acidulated with acetic acid and boiled until the sulphur which separates is collected in a mass. The hot fluid is filtered by means of a filter pump, washed with hot water, 10 c.c. of ten per cent. hydrochloric acid added, and the filtrate evaporated in a porcelain dish to 10 c.c. The filtrate should be allowed to stand for two hours, during which time the uric acid will separate, while the purin bases remain in the solution. The uric acid is then filtered off, using a small filter, and washed with water containing sulphuric acid until the filtrate and wash water together amount to 75 c.c., after which the filter paper and its contents are oxidized by the Kjeldahl method. The nitrogen obtained should be multiplied by three to give the uric acid, and three and one-half milligrams are added to the total to correct for the uric acid which has gone through the filter in the 75 c.c. of wash water and filtrate.

In the filtrate the purin bases can be determined by precipitating with copper sulphate. The fluid is rendered alkaline with sodium hydrate, then faintly acid with acetic acid, warmed to 70° C., and 1 c.c. of a 10 per cent. acetic solution and 10 c.c. of the suspension of manganese dioxide added to the fluid in order to oxidize the small amounts of uric acid which may still remain in the filtrate. The whole is then shaken for one minute, 10 c.c. of sodium bisulphite solution added, which will dissolve the excess of the manganese dioxide, and then 5 c.c. of the 10 per cent. copper sulphate solution. The fluid is boiled three minutes. The precipitate is filtered off through a filter, washed with hot water, and the nitrogen determined by the Kjeldahl method. No factor can be obtained for the nitrogen of the purin bases because the composition and proportion of these substances in the urine is variable and not determined by the process, so that the results must be expressed in terms of nitrogen.

## PROTEIDS IN THE URINE

When we speak of proteids in the urine we include a considerable group of substances of quite varying chemical positions. The same is true of the word albumin as used in a clinical sense; the term is applied loosely to include a number of related substances not strictly albumins. For a clear understanding, then, of the nature of the reactions used in determining the presence or the absence of the albumins in the urine, a slight résumé of the classification and the general reactions of these substances is necessary.

In the urine we may find mucin and nucleo-albumin, compounds chiefly interesting from the point of view that their reactions complicate the reactions of the other proteids. Serum albumin may be present, and usually with it one of the globulins. Of these, fibrinoglobulin and euglobulin are especially abundant in febrile albuminuria. In advanced chronic nephritis pseudoglobulin may be present in large quantities.[1]

Besides these, in certain cases, two varieties of albumose may appear in the urine—one very rarely, as the so-called Bence Jones' albumose or proteid; and the other, one of the normal albumoses of digestion, a secondary or deuteroalbumose. Peptone is not found except as a product of the digestion of an albumose by the pepsin always present in the urine, and as so found is always in such small quantities that it escapes detection by the ordinary methods.[2] Fibrin and hæmoglobin are also occasionally present, and very rarely bodies of the histon and nucleohiston group.[3]

### COLOR REACTIONS OF THE PROTEIDS

**Biuret Reaction.**—If an excess of strong NaOH is added to a soluble proteid and then a few drops of very dilute copper sulphate solution, a bluish or violet color will appear in the case of a proteid, or a pure pink with albumoses, peptones, and histons. It is important to avoid an excess of the copper salt, as the blue color masks the reaction. The formation of this color is due to the presence in the proteid molecule of a substance containing two $CONH_2$ groups united to a C or N atom, the simplest and best known of these being biuret, $HN\begin{matrix}\diagup CONH_2\\ \diagdown CONH_2\end{matrix}$; which can be obtained by heating urea. The sensitiveness of this reaction is very great, as it will show one part of peptone in 100,000 of water. It is less sensitive to serum albumin than to albumose or peptone, as the former has first to be split up by the alkali in order to give the reaction. The biuret reaction is given by urobilin (see under Methods of Testing for Albumose in the Urine).

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[1] For further details see *Calvo:* Zeit. f. klin. Med., Bd. li, 1904, p. 508, and *Oswald:* Beit. z. chem. Phys. u. Path., Bd. v, 1904, p. 234.

[2] See, however, *Ito:* Deut. Arch. f. klin. Med., Bd. lxxi, 1901, p. 29, who claims to have found true peptone in the urine in pneumonia.

[3] *Krehl* u. *Matthes:* Deut. Arch. f. klin. Med., Bd. liv, 1895, p. 501; also *Kolisch* u. *Burian:* Zeit. f. klin. Med., Bd. xxix, 1896, p. 374.

**Xanthoproteic Reaction.**—On warming a proteid with strong nitric acid a deep yellow color is produced which becomes reddish brown on the addition of NaOH, and orange with $NH_3$.

**Millon's Reaction.**—If a proteid is boiled with Millon's reagent a brick red color and also a red precipitate will appear in the solution. The reaction shows that the proteid molecule contains an aromatic substance in which one H of the benzol group has been replaced by an OH. Phenol is a good example of such a substance, and in the case of the proteid the reaction is due to the presence of tyrosin in the molecule of the proteid.

**Sulphur Reaction.**—If the solution of a proteid is boiled with Nylander's solution the mixture will be darkened by the formation of bismuth sulphide. The proteid must therefore be removed from urine before testing for sugar with Nylander's solution. The sulphide is formed by splitting off $H_2S$ from the proteid molecule.

The reaction may also be carried out with lead, using a few drops of plumbic acetate in the solution of proteid to be tested, and then adding enough sodium hydroxide to dissolve the precipitate of lead hydroxide which forms. The mixture blackens when heated, from the formation of lead sulphide. The reaction is due to the presence of sulphur in the proteid molecule, part of which exists as cystin.

**Carbohydrate Reaction.**—The carbohydrate group in the proteid molecule can be demonstrated by adding to a solution of a proteid a few drops of an alcoholic solution of $\alpha$-naphthol and then some strong sulphuric acid. A violet color will be produced, which becomes yellow on the addition of alcohol, ether, or NaOH (Molisch's test). The strong sulphuric acid acting on the carbohydrate group produces furfurol, which gives the color with $\alpha$-naphthol. This test will also show the carbohydrates normally present in the urine.

**Reaction of Adamkiewicz.**—The proteid solution is mixed with one volume of concentrated sulphuric acid and two volumes of glacial acetic acid. A reddish violet color is produced which is due to glyoxylic acid which contaminates commercial acetic acid reacting with the proteid.[1] It is an evidence of the presence of a member of the indol group, especially indol-amino-propionic acid or tryptophan.

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[1] *Hopkins* and *Cole:* Proceedings of the Royal Society, vol. lxviii, 1901, p. 21; also Jour. of Physiol., vol. xxvii, 1902, p. 418.

None of the color tests given above are of great importance for the clinical recognition of proteids except the biuret test which is used to identify albumose, and the sulphur reaction which is occasionally strongly given by the Bence Jones' albumose.

### PRECIPITATION REACTIONS

The practical tests for the presence of albuminous substances in the fluids of the body, especially in the urine, are based upon the precipitation of the albumin by reagents or by reagents plus the coagulating action of heat. These reactions are exceedingly delicate and are not interfered with by the other bodies in the urine. The precipitation reactions which are used are those with nitric acid, in which the precipitation is due to the insolubility of the acid albumin formed, in an excess of nitric acid; the precipitations with picric, trichloracetic, sulphosalicylic, and acetic acid, the latter precipitating nucleo-albumin without the addition of potassium ferrocyanide, but albumin only after the addition of this substance.

Another form of precipitation is that produced by the heavy metals which form bulky, insoluble compounds with the albumins.

The heat precipitations require certain conditions: the solution must be acid, and there must be a certain sufficient quantity of neutral salts present. The details will be discussed under the head of the individual tests.

### CLINICAL TESTS FOR THE ALBUMINOUS SUBSTANCES IN THE URINE

**Clearing the Urine.**—Before applying any of these tests it is necessary that the urine be perfectly transparent—a condition often impossible to obtain from a cloudy sample of urine by simple filtration through filter paper, as the bacteria and the finer precipitates readily pass through paper designed to retain fine chemical precipitates. Filtration through a tight plug of fine asbestos filtering fiber will often clear up a cloudy urine, but occasionally it is necessary to resort to other means. The best of these is to shake up the urine with either dry, powdered magnesium oxide or carbonate or fine silicious earth or sawdust, and then filter the mixture through filter paper of good quality, such as Schleicher and Schüll's No. 597. The bacteria and fine precipitates are entangled in the powder and retained on the filter.

A small quantity of the albumin is removed from the urine by this same mechanical process, but the loss is counterbalanced by the ease with which the remainder of the albumin can be detected. Clearing by means of lead acetate is inadmissible, as the lead carries down all of the albumin if the latter be present in small amounts. Centrifugalization of the urine will often clear it sufficiently to permit the albumin tests to be carried out.

## THE MUCIN-LIKE SUBSTANCES IN THE URINE

The urine contains a number of substances of differing chemical constitution which possess the common physical property of forming a gelatinous, stringy mass similar to that formed by the mucus of the saliva. These substances are present in normal urine in very small quantities, and are chiefly important because they are likely to be mistaken for albumin.

**True Mucin.**—This compound exists in the urine in solution and also in the form of a very light, gelatinous precipitate, which can be observed in normal urine, after a few hours' standing, as a faint cloud floating about the middle of the column of fluid—the so-called nubecula. The mucus which forms part of this nubecula is derived from the mucous cells of the urinary passages.

This form of proteid is precipitated in pure form by acetic acid, as is the nucleo-albumin mentioned in the next paragraph, but it is distinguished from the latter by the fact that it is easily soluble in a slight excess of the acid. It is not precipitated by boiling the acidulated solution. In the urine it is kept in solution, after the addition of the acid, by the salts present, so that it is not usually precipitated.

Mucin is also precipitated by inorganic acids, by trichloracetic and sulphosalicylic acids, and by Esbach's reagent, but is easily soluble in an excess of any of these reagents, hence does not interfere with the ordinary reactions for albumin. Even mercuric chloride does not precipitate mucin, the only metal capable of so doing being lead. Mucin is distinguished chemically from the nucleo-albumins by the fact that it contains no phosphorus, but a substance which reduces Fehling's. The mucin must be previously heated with dilute hydrochloric acid to obtain the reduction test. The detection of mucin in the urine has little clinical importance.

**The Nucleo-Albumins.**—Normal urine contains small traces of serum albumin, usually in combination with organic acids, several of which exist in the urine in very small quantities. These acids are nucleinic, chondroitin-sulphuric, and taurocholic acid. The first two are probably derived from the kidney epithelium, the last from the bile. The second named is most abundant in normal urine, the taurocholic in icteric urine.

As these three compounds with albumin and globulin exist together in the urine in varying proportions, and yet clinically are considered together, it is easy to see that the chemical properties of the group may be variable, and this may account for the confusion which exists concerning the exact reactions of these bodies. The amount of albumin or globulin normally present in the urine is insufficient to combine with all the acid present; so that when under pathological conditions the albumin content of the urine is increased, there is a corresponding increase in the amount of these mucoid substances. The chemical reactions of these compounds as a group are as follows:

1. Dilute the urine with three times its bulk of water and acidulate strongly with acetic acid. A cloud on standing is an evidence of nucleo-albumin. The dilution is for the purpose of avoiding the action of the salts of the urine in keeping the nucleo-albumin in solution. The precipitate remains on boiling the urine, hence the danger of confusing the nucleo-albumin with serum albumin.

2. If the urine is layered over cold, strong nitric acid, as in testing for albumin, a faint ring will appear about one centimeter above the surface of contact of the acid and the urine, if the latter contains nucleo-albumin.

The final decision concerning a precipitate in urine produced by acidulating with acetic acid must be made by chemical means, for recent investigations have shown that this precipitate may very probably be composed largely of euglobulin with only traces of nucleo-albumin and fibrinoglobulin. The previous identification of the nucleo-albumin was made partially upon the phosphorus content of the precipitate, but Oswald[1] has found that this phosphorus content is extremely small and variable, and is probably due to traces of nucleo-albumin and lecithin carried down by the euglobulin precipitate.

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[1] Beit. z. chem. Phys. u. Path., Bd. v, 1904, p. 234. *Matsumoto:* Deut. Arch. f. klin. Med., Bd. lxxv, 1903, p. 398.

3. The determination of the reducing power and phosphorus is as follows:

A considerable quantity of the urine—about 1,000 c.c.—is slightly acidulated with acetic acid and the precipitate filtered off, washed with water, and rubbed up in a mortar with a little sodium carbonate solution.

It may be necessary to add also a very small quantity of sodium hydrate to complete the solution of the precipitate. Filter and precipitate again and wash with water. This is to remove the phosphates which are carried down by the precipitate and would interfere with the final tests for phosphorus. Divide the final precipitate into two portions. One half is shaken up with about 25 c.c. of HCl diluted one to three, the mixture boiled gently for about ten minutes, allowed to cool, rendered alkaline with NaOH, and a little dilute $CuSO_4$ added. Boil and allow to cool and settle. If mucin is present in the precipitate there will be a red deposit of copper oxide at the bottom of the flask.

The other half of the precipitate is rubbed up in a mortar with a little strong alcohol and heated on a water bath. Filter and wash with strong alcohol. The precipitate is placed in a flask and shaken up with ether, allowed to stand for some time, filtered, and the dried precipitate fused with 30 parts of a mixture of sodium carbonate, one part, potassium nitrate, three parts. The fused mass is dissolved in nitric acid and boiled; then add gradually about 5 c.c. of a solution of ammonium molybdate. A yellow precipitate indicates the presence of phosphorus, which is present in considerable amount in all the nucleo-albumins.

The nucleo-albumins occur in normal urine only in traces. Larger quantities are to be found in the urine of the new-born; in the urine after overexertion; in inflammation of the mucous membrane of any part of the urinary tract; in leukæmia; in acute diseases, especially such as attack the kidney (scarlatina or diphtheria); after injuries to the kidney or alterations in the renal circulation; after chloroform inhalations. These compounds are present very abundantly in the urine of persons suffering from jaundice. Clinically an increase in nucleo-albumin indicates only an irritation or inflammation of the urinary tract, as the amount contained in the blood is very small.

The thick, ropy substance which is found in the urine of patients suffering from cystitis is chiefly a nucleo-albumin extracted from the leucocytes of the pus which collects in the bladder, and when the urine undergoes alkaline fermentation the nucleoalbumin is softened and rendered stringy by the alkali and the sodium chloride present.

### ALBUMIN IN THE URINE

In speaking in a clinical sense of albumin in the urine the term so used includes serum albumin and globulin, both of which are proteid bodies giving a coagulum when the urine containing them is boiled. Under ordinary circumstances no attempt is made to distinguish these two closely related substances, and, as they almost always appear together, such a distinction has seemed to have but little practical value. Of late, however, an attempt has been made to show that the amount of globulin is greater in proportion to the albumin in cases of nephritis in which there is a considerable lesion of the renal epithelium, and also in amyloid degeneration of the kidney.

The tests which follow make no distinction between albumin and globulin.

### QUALITATIVE TESTS FOR ALBUMIN

**Heat Test for Albumin.**—One of the most satisfactory and reliable tests for albumin in the urine is based upon the coagulation and precipitation of the albumin by heat in the presence of a small amount of acid and sufficient neutral salts.

*With Nitric Acid.*—The clear urine is placed in a narrow test tube and the upper portion of the fluid heated to boiling. One or two drops of strong nitric acid are added; if no precipitate appears, or if the precipitate redissolves on shaking, add two or three drops more. A white or brownish flocculent precipitate is albumin. The hot fluid is much darker in color after the acid is added. This is due to the breaking up of indoxyl and skatolcarbonic acids and liberation of the pigments. Only the upper portion of the fluid should be heated, then, when very small quantities of albumin are present, the faint cloud which they produce can be easily noted by comparing with the clear unheated urine below. The tube should be held against a dark background in such a way that the light falls downward through the fluid and toward the observer.

This reaction is an excellent one, but certain sources of error must be clearly held in mind. A flocculent precipitate formed before the addition of the acid may be calcium phosphate, which is identified by its easy solubility in an excess of acid. If the urine is of low specific gravity and contains but a small amount of salts, the precipitate due to albumin may not form, or it may redissolve in the hot fluid unless a very considerable excess of acid be added; in this case, it is well to add to such a urine 1 c.c. of a saturated (about 36 per cent.) solution of sodium chloride to each 5 c.c. of urine.

The urine should *not* be heated after the addition of the acid, as the coagulated albumin is soluble in the hot acid; indeed, this is one of the reasons for the fact that this test is not extremely delicate, as very faint traces of albumin may be dissolved on the addition of the acid. Neither albumose, nucleo-albumin nor mucin is precipitated so long as the fluid remains hot.

Uric acid or the urates may give rise to precipitates which are crystalline and not flocculent, and which do not appear if the urine be diluted, previous to the test, with three parts of water or sodium chloride solution.

Resinous bodies may be excreted in the urine after the administration of turpentine, copaiba, sandalwood oil, Peru or tolu balsam, and occasionally give precipitates which are best distinguished from proteids by shaking the *cooled* urine with petroleum benzine, when the precipitate due to the resins or oils will dissolve; that due to the albumin will remain.

Urines deeply colored with bile pigments occasionally give a precipitate of biliverdin, which is of a green color and soluble in alcohol and thus distinguishable from coagulated albumin.

*With Acetic Acid.*—The reaction of the urine is tested, and, if alkaline, it is to be rendered slightly acid with a few drops of *2 per cent. acetic acid.* The urine is then heated in the upper portion of the test tube to the boiling point, leaving the lower part of the fluid cool as a control. A white, flocculent precipitate is probably coagulated albumin, but it may be partly composed of nucleo-albumin. If, however, one-fifth volume of a saturated NaCl solution is added with a few drops more acid and the whole boiled again, the nucleo-albumin and the mucin are not precipitated.

The great source of error in this method lies in the use of too much acetic acid, which converts the albumin present into an easily soluble acid albumin which is not coagulated on boiling.

The albumoses are not precipitated unless there is a considerable quantity of sodium chloride present in the urine; if this is the case the albumose precipitate becomes visible only when the urine is cooled, and hence after the coagulated albumin has been removed by filtering the fluid while still hot. The albumose precipitate can again be made to disappear from the cooled fluid by reheating. The Bence Jones' proteid coagulates when the fluid reaches about 60° C. and then dissolves as the temperature rises. The resins mentioned in the preceding section are detected in the manner there described.

**Heller's Test.**—A few cubic centimeters of concentrated (not fuming) nitric acid are placed in a test tube and the urine to be tested is allowed to run slowly down the side of the tube, so as to flow gently over the surface of the acid and form a distinct layer on it with as little commingling of the two fluids as possible. The best way to accomplish this is to use a medicine dropper with a long tube and allow the urine to flow out slowly by gently pressing on the bulb.

If an ordinary pipette is used, the test tube should be held at an angle of 45° and the pipette placed just inside the mouth of the test tube with its tip resting against the wall of the tube. This will break the force of an accidental spurt of urine from the pipette and prevent its mixture with the acid except by flowing slowly down the wall of the tube. The finger closing the upper end of the pipette should be dry, and the fluid escapes best if the pipette is slowly rotated between the thumb and fingers, so as to allow a little air to enter under the tip of the finger closing the pipette.

Some recommend that the urine should be placed first in the tube and the acid run under it by means of a pipette, the heavy acid sinking to the bottom. The first method is the preferable one.

The action of the nitric acid is to precipitate the albumin as acid albumin, and since the latter is insoluble in the great excess of acid present at the point of contact, a white ring of proteid is formed. Albumose and the resins are precipitated by this method, also nucleo-albumin, though the ring due to the latter is about one-half to one centimeter above that due to albumin. Mucin is also precipitated, but is dissolved by the excess of acid present, so that it does not contribute to the ring due to albumin. Uric acid and urea nitrate may be precipitated from concentrated urines, but are easily recognized by their crystalline nature. The precipitate due to the presence of the resins can not be easily differentiated from that produced by acid albumin, especially by the addition of alcohol, as is generally recommended, since the acid albumin is also somewhat soluble in alcohol and the ring disappears when the fluid is shaken. The differentiation had best be made by one of the other tests which does not precipitate the resins—for example, sulphosalicylic acid.[1]

**Robert's Test.**—The solution is composed of one part of strong nitric acid and five parts of a saturated solution of magnesium sulphate. It is used in precisely the same manner as the nitric acid in the Heller's test, and is subject to the same disadvantages by its precipitation of the nucleo-albumins and mucin, the latter not being dissolved as quickly as with the pure nitric acid. It is much more sensitive than Heller's test. The advantages in its use are that the solution is not so corrosive nor so dangerous to handle as pure nitric acid. The test should not be allowed to stand longer than five minutes or a ring will be produced in normal urine.

**Ferrocyanide and Acetic Acid.**—To 10 c.c. of urine add 5 drops of strong acetic acid. If any precipitate is produced it is due to nucleo-albumin and should be filtered off. Then a few drops of a 5 per cent. solution of potassium ferrocyanide are added. An excess will dissolve the albumin precipitate. As the reagent and the urine can not be kept in distinct layers, it is advisable to hold a comparison test tube of the same urine together with that to be tested before a black cloth, as otherwise the slight opalescence due to a trace of albumin may escape observation. Albumose and nucleo-albumin give precipitates. The former dissolves on heating the mixture; the latter is best removed by adding to the urine a very few drops of dilute lead acetate solution and filtering. Great care must be taken not to add an excess, as this will remove the albumin also.

**Spiegler's Test.**—The reagent consists of 40 grams of mercuric chloride, 20 grams of tartaric acid, 1,000 c.c. of water, 100 c.c.

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[1] *Kowarsky* (Lehrbuch d. klin. Untersuchungsmethoden, Bd. i, p. 69) states that the resins are also precipitated by sulphosalicylic acid, but with this the writer can not agree.

of glycerin. The urine is *strongly* acidified with acetic acid and layered over the reagent. If the urine is of very low specific gravity it is necessary to add some sodium chloride solution, or the albumin will not be precipitated. The albumose precipitate dissolves on heating and does not reappear to any extent on cooling. This test for albumin is one of the most delicate, and should be used in all cases where the detection of a trace is important; but its very delicacy is a danger, for it will sometimes show a well-marked ring when there is no abnormal albumin in the urine, but only a slight increase in the amount of nucleo-albumin present.

**Sulphosalicylic Acid.**—This substance can be used in a 20 per cent. solution, or a small fragment of the acid can be dropped into a test tube of urine, and as it dissolves will give a white ring which, on shaking, spreads throughout the fluid as an opalescent cloud or a bulky precipitate, depending upon the amount of albumin present. Albumose is precipitated, but not uric acid nor the resins; the albumose disappears on heating, reappearing on cooling.

**Trichloracetic Acid.**—A saturated aqueous solution of the acid is used over which the urine is to be layered. The solution can with advantage be rendered more dense by saturating it with magnesium sulphate; the mechanical difficulties encountered in layering two fluids of slightly differing specific gravity are thus lessened. Albumose is precipitated imperfectly and dissolves on heating. In very dense urines the urates may be precipitated, but this source of error can be easily eliminated by dilution of the urine. Mucin is precipitated, but dissolves as the acid diffuses.

The delicacy of all these layer tests is considerably increased if the point of contact between the urine and the reagent is sharp. A convenient apparatus for this purpose is a small U-shaped tube with a funnel at each end, which is sold under the trade name of "horismascope." The urine to be tested is filtered into the larger funnel, acidulated if necessary, and then the reagent is poured into the small funnel. The reagent, being of higher specific gravity than the urine, flows down the capillary tube and forms a layer under

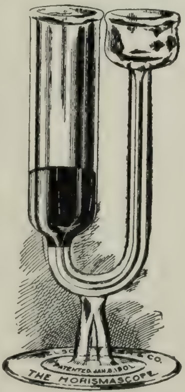

FIG. 140.—HORISMASCOPE.

the urine. After standing for one or two minutes the apparatus is examined in a strong vertical light. The slightest opacity at the contact point of the two fluids is easily seen.

### DELICACY OF THE VARIOUS TESTS

The sensibility of the various tests is dependent upon a great many circumstances, which vary in different specimens of urine and make a test like Spiegler's, for instance, sensible to one part of albumin in 250,000 when the urine contains an abundant amount of sodium chloride, while in a urine of very low specific gravity the delicacy is much less. Allowing for these sources of error, however, the tests may be roughly grouped as follows: The most sensitive is Spiegler's, closely followed by trichloracetic and sulphosalicylic acids, then Robert's, the boiling test with acids, the ferrocyanide test, Heller's, and finally the boiling with neutral salts and addition of nitric acid.

### QUANTITATIVE TESTS FOR ALBUMIN

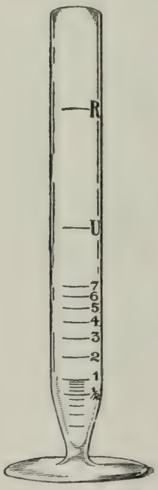

Fig. 141.—Esbach Tube. Improved form.

**Esbach Test.**—The most useful quantitative test for albumin, which is simple and does not require the use of a balance, is that bearing the name of Esbach, and based upon the precipitation of the albumin by a solution of picric and citric acids in a tube which is graduated empirically to show the number of grams of albumin per liter of urine. The tube is to be filled to the mark U with the urine to be tested. The urine must be acid, must have a specific gravity of not over 1.008, and must not contain more than 4 grams of albumin to the liter. It is to be acidulated with acetic acid if alkaline, and diluted to the required degree. After filling the tube to the proper height with urine, the reagent is to be added to the mark R.

The reagent contains 10 grams of picric and 20 grams of citric acid to 1,000 c.c. of water.

The tube is then corked and slowly inverted several times to mix the two fluids, being careful not to cause any foaming, which prevents the deposition of the albuminous coagulum. The whole is then set aside for twenty-four hours in a cool place and the amount of albumin read off from the graduation.

The influence of temperature on the height of the deposit is very marked; it is much more bulky in cold weather than in hot, and the error may be as much as 50 per cent. of the whole, so that it is important to keep the tube as nearly as possible at a constant temperature of 15° C. during a series of determinations. The results are, however, sufficiently accurate for clinical purposes, and the instrument must remain the only simple means for the approximate estimation of albumin in the urine.

**Gravimetric Method.**[1]—One hundred c.c. of the urine to be tested is filtered to remove all epithelial cells or crystals, and in case the reaction is not distinctly acid, a drop or more of acetic acid is added. The urine is then placed in a 250 c.c. beaker and warmed gradually over a water bath for half an hour, until the albumin has formed a coarse flocculent precipitate. The addition of a couple of drops of acetic acid may be necessary if the precipitate does not become flocculent. The urine is now filtered through a small ash-free filter which has been dried at 110° C., or through a Gooch asbestos filter dried at the same temperature. Great care should be taken to collect all of the albumin which may be adherent to the walls of the beaker by means of a glass rod covered with soft rubber tubing, and to add it to the contents of the filter. The precipitate is then washed with hot distilled water until the wash water is free from chlorides when tested with silver nitrate.

The filter is then filled twice with strong alcohol and twice with ether, then dried at 110° C., until the weight is constant. If the amount of albumin is large it is necessary to know the quantity of ash which it contains, as this interferes considerably with the accuracy of the result. The dried filter and its contents must therefore be carefully heated in a crucible until all organic matter has been driven off. The weight of the ash subtracted from the weight of the albumin gives the amount of albumin present in the urine.

Numerous other methods have been suggested for the quantitative determination of albumin, none of which approach in accuracy the gravimetric method, nor in convenience that of Esbach.

Purdy[2] recommends the use of an electric centrifuge and graduated tubes to determine the albumin quantitatively. The tube is filled with 10 c.c. of urine, 3 c.c. of a 10 per cent. solution of potassium ferrocyanide, and 2 c.c. of 50 per cent. acetic acid, and after standing for ten minutes is centrifuged for three minutes at a speed of 1,500 revolutions per minute in a centrifuge whose radius is 6.75 inches. The accuracy of the method depends entirely upon the speed and size of the centrifuge. These instruments when first purchased will easily run 1,500 revolutions, but after a few months of use they usually fall to a thousand. Even with a centrifuge running exactly the required number of revolutions the writer has found that the deposition in the tube takes place irregularly and that the results are not as accurate as those obtained by the Esbach method, though they have the special convenience of being obtained in a few minutes.

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[1] *Salkowski:* Practicum, second edition, p. 254.

[2] Practical Urinalysis, sixth edition, Philadelphia, 1901.

Determinations of the quantity of albumin by polarization or determination of the refractive index before and after boiling, and by determining the specific gravity under the same conditions, have no advantage over the gravimetric method.

Precipitation methods, such as that of Wassilyew, which consists in titrating the urine with a standard solution of sulphosalicylic acid, have been shown by Vogel [1] to be quite inaccurate.

If only an approximate estimation of the albumin is necessary, it may be remembered that a urine which boils solid contains from 2 to 3 per cent. of albumin. If the test tube after boiling is half filled with the coagulum, about 1 per cent. is present, one-third of the column is approximately 5 parts to the thousand; one-tenth of the column, 1 part to the thousand. A faint clouding is less than 1 part in ten thousand.

### METHODS OF REMOVING ALBUMIN FROM THE URINE AS A PRELIMINARY TO OTHER TESTS

1. The acid urine from which the albumin is to be removed is heated on a water bath or over a flame until it just begins to boil. Neutral or alkaline urine must be faintly acidified with acetic acid. If the separation of the albumin does not take place in large masses the addition of a few drops of acetic acid will often facilitate the separation. Occasionally it is necessary to add about 10 c.c. of a saturated solution of sodium chloride to urines which contain but a small amount of neutral salts. Excess of

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[1] Proceedings of the N. Y. Path. Society, N. S., vol. iv, 1904, p. 38.

acetic acid forms an acid albumin and should therefore be very carefully avoided. The urine when filtered from the coagulum must be perfectly clear, and not give a precipitate with sulphosalicylic acid or ferrocyanide and acetic acid. This method of removing albumin is sufficiently complete for the urea and sugar tests, but urine so treated can not be tested directly for the albumoses because the boiling of the acid is likely to break up some of the albumin, which would then react with the biuret test. If albumose tests are to be applied, the following method is preferable :

2. Sodium acetate and ferric chloride are added to the urine until the fluid is colored a bright red. If after this addition the reaction is strongly acid, dilute sodium hydrate should be added until the urine becomes neutral or faintly acid. The fluid should then be heated to boiling, during which process the albumin will separate out in a coarse flocculent precipitate. The filtrate should not give a cloud with potassium ferrocyanide and acetic acid, nor should it give a strong blue color, which would indicate that traces of iron salts still remain.

### CLINICAL SIGNIFICANCE OF ALBUMINURIA

Traces of albumin are present in every normal urine, but in such small quantities that they escape detection by the ordinary clinical tests. Only in using reagents such as Spiegler's, sulphosalicylic acid, and Robert's solution, are we likely to obtain a reaction in normal urine.

When albumin is found in amounts larger than normal in the urine the first question to be decided is the site of its origin. If the albumin comes from pus or an exudate from any portion of the genito-urinary tract, or from hæmorrhage from any part of the tract, it must be traced to this local cause. This is often a most difficult matter, as, for instance, in deciding the question as to whether the albumin in a given specimen is derived from a cystitis from which the patient is suffering, or whether the patient is also suffering from a nephritis which is responsible for a portion of the albumin. It is to be remembered that the treatment of a bladder lesion may increase the albumin, and massage of the prostate is occasionally followed by a trace of albumin in the urine.

The decision can only be made after a long and careful search for casts in the urine, rendering the latter acid, if possible, by the use of drugs such as urotropin, and centrifuging the freshly passed urine in order to avoid the solvent action of the alkaline fluid upon the casts. It may be necessary to wash out the bladder and to collect the urine immediately, in order to reduce as much as possible the amount of the thick pus, which is likely to so obscure the field of the microscope as to render the finding of the casts a difficult matter. If casts are found, a portion of the albumin is evidently dependent upon a kidney lesion. If the ratio between the albumin content of the urine by Esbach and the leucocytes per c.mm. is as 1 to 60,000 or 80,000, the albumin may be considered as derived from the pus. If the leucocytes are fewer in number and the albumin is over 0.1 per cent. there is probably a coincident albuminuria.[1] It is often very difficult, however, to obtain a satisfactory enumeration of the leucocytes.

If the albuminuria has been definitely traced to the kidney, what does the presence of this proteid indicate clinically? It may be safely assumed that it is an evidence of a pathological condition, though not always indicative of a sufficient change in the structure of the kidney to warrant the assumption of a nephritis. Renal albuminuria is always dependent upon some lesion of the tubular or glomerular epithelium sufficient to permit the escape of the serum albumin and globulin from the vessels into the urine, and such a kidney is not normal. This is the point of view assumed by the life insurance companies, and is based upon the study of a very large number of cases.

The various types of albuminuria are as follows:

1. "Functional," "cyclic," and "transitory" albuminuria are all to be regarded as due to slight alterations in the kidneys, without, perhaps, sufficient change to be called a nephritis. They are chiefly seen in anæmic and poorly nourished children, and in adults after very severe exercise, cold baths, dietary indiscretions, etc. Casts are usually absent and the amount of albumin is small. Such cases often go on later in life to show distinct evidences of nephritis. Evidently the kidneys of such patients are notably insufficient, and though not necessarily the site of a nephritis, should be regarded as very likely to develop that condition.

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[1] *Goldburg:* Cent. f. d. med. Wissen., 1893, p. 593. *Hottinger:* ibid., p. 257.

2. Dietetic Albuminuria (*Alimentary albuminuria*).—Traces of albumin may be found in the urine after a meal containing large amounts of proteids, and Inouye,[1] working with the precipitin method, finds that an alimentary albuminuria due to the presence of ovalbumin in the urine may be produced by three to six raw eggs taken on an empty stomach. Others have also shown [2] by the precipitin reaction, that in the urine of some patients suffering from intermittent albuminuria, beef serum could be demonstrated. An explanation of this seems to be that an imperfect digestion of the serum allows some of that substance to escape and to be absorbed in an unaltered form. If the gastric digestion is imperfect this is possible, for tryptic digestion does not always destroy the substance which incites the precipitin reaction. It has, however, been pointed out that inasmuch as the precipitin reaction is not specific for albumin but only for some precipitable body which may not be albumin, the only fact that the above experiment demonstrates is that the precipitable substance in beef serum is able to pass the kidney epithelium of patients suffering from intermittent albuminuria.

3. Albuminuria due to circulatory disturbances is seen most frequently in severe and uncompensated heart lesions as a result of the changes in the kidney from the altered blood pressure. The quantity of albumin found under these conditions is usually small and a few hyaline casts may be present. The urine can usually be distinguished from that of a true diffuse nephritis by the fact that the amount of the albumin varies with the amount of the urine, and that the latter is often small in amount and highly colored.

4. Febrile albuminuria is usually present in all forms of fevers, and is due to degenerative changes in the kidney epithelium, which may be entirely repaired, or may go on to a true nephritis with the appearance of large quantities of albumin and numerous casts.[3]

5. Toxic albuminuria is a symptom of poisoning by ether, mercury, cantharides, phosphorus, lead, arsenic, the mineral acids, etc. The change in the kidney may be of a degenerative nature

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[1] Deut. Arch. f. klin. Med., Bd. lxxv, 1903, p. 378.

[2] *Linossier* et *Lemoine:* C. R. Soc. de Biol., vol. liv, 1903, p. 415.

[3] See an interesting monograph by *Hallauer:* Ueber Eiweissausscheidung im Fieber, Verhandlungen der phys. med. Gesellschaft zu Würzburg, N. F., Bd. xxxvi, 1904.

or it may be connected with circulatory disturbances. The globulin quotient in acute toxic lesions is often high, but there is no connection between the relative proportions of these substances and the efficiency of the kidneys as measured by the depression of the blood freezing-point.[1] Nucleo-albumin is usually present.

6. The albuminuria seen in connection with the severe anæmias and leukæmia is probably dependent upon the degenerative changes so common in the organs of patients with such disease.

7. The albuminuria of nephritis is very variable. In acute nephritis the amount is very large, and in the hæmorrhagic forms is increased by the direct transudation of blood from the vessels. The amount lost from the body may rise as high as 20 grams in twenty-four hours. The amount is a fair index of the severity of the disease.

In the chronic parenchymatous forms the amount is also large, and may even exceed that passed in the acute type, but, as a rule, 5 grams a day is a large amount. The urine is diminished in amount and has a high specific gravity. Occasionally very large amounts of albumin may be seen in the course of a syphilitic nephritis.

The different types of chronic diffuse nephritis give a most variable picture. Albumin may be absent in a well-marked case and only a few hyaline casts be found to suggest the possibility of a renal lesion; and this condition may exist when the patient is close to an attack of uræmia, while during an acute exacerbation of the disease the albumin may reach as high a point as in the acute cases. Many patients live for months and years with merely a trace of albumin, which may disappear temporarily and then reappear as some change comes in the general condition of the body, or as a fresh portion of the kidney is diseased. The albumin may be more abundant at the end of the day, or after meals or after exercise, and absent in the morning urine. The amount has not the least relationship to the severity nor to the extent of the lesion.

## ALBUMOSE IN THE URINE

In the digestion of the proteids by the gastric juice a number of bodies are formed which have slightly different properties from those of the original proteid and which differ slightly among themselves. A simplified scheme of the products of pepsin digestion is given below. The actual process is probably much more complex and may go on to the stage of the amino bodies, such as leucin and tyrosin.

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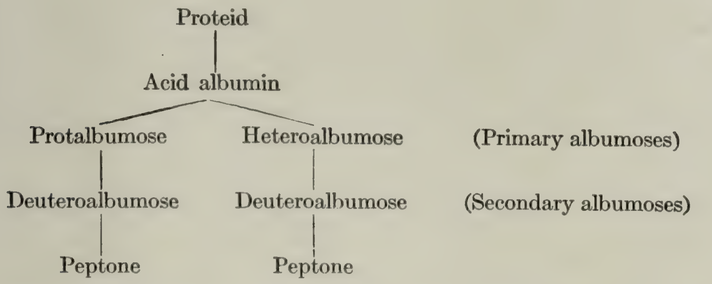
[1] *Cloëtta:* Arch. f. exp. Path. u. Pharm., Bd. xlviii, 1902, p. 223.



The substances which appear in the urine are the primary and secondary albumoses. Peptone as a rule is not found, or if so is due to the proteolytic action of the urinary pepsin. Ito[1] claims, however, to have found true peptone in acute phthisis and pneumonia.

### BENCE JONES' PROTEID

In the urine of persons suffering from multiple myeloma or leukæmia a proteid has been found which differs in its reactions from serum albumin and globulin, and has been generally assumed to belong to the albumose group of proteids. It has been shown quite recently, however, that this body is probably not a true albumose but is of proteid nature.

The reasons which have been adduced against the Bence Jones' proteid belonging to the albumose group are its complete coagulability when heated under certain conditions, and the ease with which it becomes insoluble after treatment with strong alcohol; the Bence Jones' proteid forms syntonin, while albumose does not; on digestion of the Bence Jones' proteid, primary and secondary albumoses are produced, with the exception of heteroalbumose. The latter substance may, however, be absent from the proteid molecule, as for example casein, which does not contain this group.

The amount of proteid which may be found in the urine is variable, quantities as high as 6 per cent. having been seen. Casts are usually not found, nor advanced changes in the kidneys.

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[1] Deut. Arch. f. klin. Med., Bd. lxxi, 1901, p. 29.

About thirty cases of this disease have been described in the literature.[1]

The reactions of the Bence Jones' proteid are as follows:

1. On heating the urine a faint opalescence may be noted at 50° C., while a precipitate begins to form at about 58° C., which continues to increase in amount until a point near 70° C. is reached. A slight clearing of the fluid then begins, and by the time the boiling point is reached a very large portion of the cloud has disappeared. Usually, however, a slight opalescence remains. If cooled, the precipitate reappears in the fluid. It clings more to the walls of the test tube than does the albumin coagulum.

2. Twenty-five per cent. nitric acid precipitates the proteid at room temperatures. The precipitate is partially soluble on boiling, and if the fluid is again cooled the precipitate reappears.

3. Thirty per cent. acetic acid causes no precipitation.

4. Millon's reaction and the biuret reaction are given strongly.

5. An abundance of lead blackening sulphur is often but not always present,[2] as shown on heating some of the urine which has been rendered strongly alkaline after the addition of a few drops of plumbic acetate.

6. On the addition of 2 volumes of 96 per cent. alcohol the proteid is completely precipitated.

7. Two volumes of saturated sodium chloride solution produce no clouding. If acetic acid is added the precipitation is quite complete.

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[1] *Magnus-Levy:* Zeit. f. phys. Chemie, Bd. xxx, 1900, p. 200; *Hamburger:* Johns Hopkins Hosp. Bull., 1901, p. 38; *C. E. Simon:* Amer. Jour. Med. Sciences, vol. cxxiii, 1902, p. 954; *Coriat:* ibid., vol. cxxvi, 1903, p. 631; *Weber:* ibid., p. 644; *Boston:* ibid., vol. cxxv, 1903, p. 658; *Askanazy:* Deut. Arch. f. klin. Med., Bd. lxviii, 1900, p. 34 (reports a case of Bence Jones in a patient with lymphatic leukæmia probably of a myelomatous type). See also *Cohnheim:* Chemie d. Eiweisskörper, Braunschweig, 1904, p. 172.

[2] See on this point *Lindemann* (Deut. Arch. f. klin. Med., Bd. lxxxi, 1904, p. 114), who found no more lead blackening sulphur in a specimen of Bence-Jones' proteid than in a corresponding amount of serum albumin. A specimen of Bence Jones' proteid examined by the writer does not show any great amount of lead blackening sulphur.

8. Saturation of the urine with magnesium sulphate at room temperature causes no precipitation.

9. The addition of two volumes of a saturated solution of ammonium sulphate causes complete precipitation.

10. Picric acid gives rise to a marked precipitate which does not dissolve on boiling the fluid.

11. Acetic acid and potassium ferrocyanide give a moderate precipitate which dissolves on heating and reappears on cooling.

### ALBUMOSURIA

The secondary or deuteroalbumoses are the albumoses commonly found in traces in the urine in about 90 per cent. of the febrile diseases. They appear also in septic conditions, and their presence in the urine of a case of meningitis is in favor of a suppurative process and against a tuberculous one. In empyema the albumose produced by the action of the leucocytes, the bacteria, and the autolytic ferments on the serum of the exudate is found in the urine in large quantities. It is present in the urine in cases of ulceration of the bowel, either typhoidal or dysenteric, less commonly in ulcers of a tuberculous origin. Phosphorus poisoning and abscesses of the liver also give rise to albumosuria. Malignant growths in any portion of the body are occasionally accompanied by albumosuria, probably due to the ulceration of the growth.

The reactions of the secondary albumoses are different from those of the primary albumoses or the Bence Jones' proteid.

Urine containing these bodies does not become cloudy on boiling; does not give Heller's test with nitric acid; gives no precipitation on saturating with sodium chloride when the reaction of the urine is neutral, but does give a precipitate when the reaction is made acid with acetic acid saturated with sodium chloride. Ferrocyanide and acetic acid give no precipitate unless the solution contains a considerable quantity of neutral salts. Trichloracetic and sulphosalicylic acids give precipitates which disappear on heating and reappear again on cooling. It is rare that deuteroalbumose is present in the urine in sufficient amount to give well-marked precipitation reactions, especially if any albumin is also present. The best test for the deuteroalbumoses is the following, which permits the detection of traces in the urine:

**Tests for Albumose.**—In urines which are light in color and which do not contain a large quantity of urobilin the following method is the best and simplest:

1. *Hofmeister-Salkowski Method.*—Fifty c.c. of urine are acidulated with 5 c.c. of HCl; then 10 per cent. phosphotungstic acid solution is added until a precipitate ceases to form. The mixture is then placed in a beaker (or a test tube if working with small quantities), and gently heated without boiling until the precipitate shrinks up into a resinous mass and collects at the bottom of the beaker.

The supernatant fluid is then poured off and the resinous mass washed with several changes of water. Some dilute sodium hydrate solution is then added, and the deep blue solution is gently warmed until it changes to a pale yellow. It is then cooled in a test tube and a very dilute solution of copper sulphate allowed to run down the side of the tube. If the two fluids are now mixed by gently shaking, a rose color will appear if the fluid contains albumose. This is the biuret reaction for albumose referred to on page 491.

If the urine contains albumin it must first be removed, as this proteid will give the same reactions. The best method of removal is to add to the urine some sodium acetate and then enough ferric chloride to give the whole a deep blood-red color. The reaction of the fluid is changed to neutral by the addition of a little sodium hydrate and the mixture boiled and filtered. To test the result add some acetic acid and potassium ferrocyanide; if there is any cloud visible the albumin has not been completely removed and the process must be repeated. The albumin may also be removed by adding an equal bulk of a 10 per cent. solution of trichloracetic acid and boiling and filtering hot. The albumoses will pass through the filter. If the urine contains mucin, nucleoalbumin, or histon, the whole must be strongly acidulated with acetic acid and allowed to stand for several hours and then filtered from the resulting precipitate. Then the albumin may be removed as above. Another method is to precipitate with a very few drops of lead acetate solution and then remove the albumin. If the albumin is removed by acidulating with acetic acid and boiling, traces of albumose are likely to be formed by the action of the acid.

If the urine contains urobilin, it is necessary to wash the precipitate produced by the phosphotungstic acid with a considerable quantity of strong alcohol until the alcohol is no longer colored yellow by the urobilin. This may be done either by shaking up the precipitate with the alcohol and then centrifuging and repeating the process, or by placing the precipitate on a filter and extracting the color by repeated washings with alcohol. The washed precipitate is then suspended in water, dissolved in sodium hydrate, and treated with the dilute copper sulphate.

2. *Method of Bang.*—Albumose can also be detected in the urine by the following procedure: Ten c.c. of the urine are heated and saturated with ammonium sulphate, which requires usually about 10 grams of the salt. As soon as the solution is complete the mixture should be heated to boiling, but long heating should be avoided, as otherwise albumoses may be formed. The precipitate consists of albumose, albumin, urobilin, and uric acid and its salts. The hot fluid is centrifuged for a couple of minutes and the residue rubbed up in a mortar with a small amount of ninety-six per cent. alcohol which dissolves out the urobilin, which can be demonstrated by a spectroscope on the addition of alkaline zinc chloride solution. The alcohol is poured off and the residue dissolved in distilled water. The solution is warmed and filtered from any cloud. Albumin, uric acid and salts remain on the filter, while the filtrate contains albumoses which can be tested by the biuret reaction.

Large quantities of heteroalbumose may be recognized, even in the presence of albumin, by acidifying the urine with acetic acid, adding an equal quantity of a saturated solution of sodium chloride, boiling and filtering hot. The filtrate is cooled in a test tube, and any opalescence or precipitate is albumose.

## CARBOHYDRATES IN THE URINE

Traces of glucose are present in all normal urines in quantities varying from 2 to 5 centigrams in twenty-four hours, or from 0.01 to 0.03 per cent. Normal urine, however, has a considerably higher reducing power than this, varying from 0.08 to 0.6 per cent. computed as glucose, one-sixth to one-half of this being due to the reduction caused by uric acid and creatinin.[1] Another portion of the reduction is due to glycuronic acid, which is always present in normal urine in the form of conjugate compounds. The reduction due to this substance may be considered as about 0.07 per cent. computed as glucose. Isomaltose may possibly also contribute to the reduction of Fehling's solution.

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[1] *Long:* Chem. Cent., Bd. ii, 1900, p. 402.

An abundant meat diet and fever increase the reducing power of the urine though no more glucose is present, while starvation diminishes the reducing action on Fehling's.

The carbohydrates and the sugars which the food contains are usually altered by the digestive juices before they are absorbed.

**Starch** is split through the stages of erythrodextrin and achroödextrin into maltose, and the latter is altered in its passage through the intestinal wall into glucose, and has only been found in the urine on a few occasions.

**Isomaltose** is a sugar derived from starch by the action of ptyalin, or amylopsin. A very small quantity is found in normal urine.[1]

**Cane sugar,** or saccharose, is split into glucose and lævulose and absorbed in these forms. Only when the amount of cane sugar ingested is over 200 grams is it absorbed into the blood as such, and this only occurs when given fasting; when given during a meal with an abundance of other food, the capacity of the digestive juices to split up the sugar and the body to absorb it is much greater. If any cane sugar reaches the circulation as such it is immediately excreted by the urine, but may be altered into glucose during its passage through the kidneys.

**Glucose** is directly absorbed by the mucous membrane of the stomach and intestine.

**Lævulose,** which is a sugar abundant in honey and ripe fruits, is absorbed unchanged. It is used to replace glucose in the carbohydrate food of the diabetic, and under these conditions is often, though not always, used in the body when the same quantity of glucose would reappear largely in the urine. Traces of lævulose are usually present in the urine of diabetics.

**Lactose,** or milk sugar, is split in the intestinal wall into glucose and galactose and thus absorbed. When lactose is absorbed directly into the blood it is excreted in the urine quantitatively. This occurs when over 120 grams are taken on an empty stomach, and also in nursing women, especially if there is any obstruction to milk secretion.

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[1] *Lemaire:* Zeit. f. phys. Chem., Bd. xxi, 1895, p. 442.

**The pentoses** are a group of sugars which occur in fruit and vegetables, coffee, tea, and milk, and in combination with some of the animal nucleo-proteids of the food. The pentose sugar, l-arabinose, is the one which has been found most often in urine after excessive eating of fruit. A small part of the pentose which is taken in from the food is used in the body, most of it is excreted in the urine. That which is used is stored up as glycogen in the liver.

### GLYCOSURIA

The most common cause of glycosuria, and that which produces the disease known clinically as diabetes, is, as is now generally assumed, an alteration in the organism which prevents the proper metabolism of the carbohydrates, the anatomical site of the lesion being most often found in the pancreas. It has been shown that many, though not all, cases of diabetes show changes in the islands of Langerhans of the pancreas, though the remainder of the gland may appear to be normal.[1]

Glycosuria, often accompanied by the symptoms of diabetes, is frequently observed after involvement of the pancreas by a new growth, or after blocking of the pancreatic duct by a calculus and subsequent atrophy of the gland.

Other forms of glycosuria which are not accompanied by the symptoms of diabetes are those due to an excess of carbohydrate in the food, the so-called alimentary glycosuria, to drugs such as phlorizin, chloroform, amyl nitrate, and after asphyxia from the inhalation of illuminating gas. After severe burns glucose may be found in the urine. Glycosuria accompanies cirrhosis of the liver quite frequently; it is often seen after severe injuries to the brain, or shock from an injury to some other portion of the body, or after an attack of apoplexy, and in many nervous diseases. Glycosuria as an accompaniment of paresis and dementia paralytica is often seen and is usually transient. Cases which persist are usually true diabetes.

Glycosuria and also diabetes are not infrequently connected with exophthalmic goitre. Glycosuria may follow thyroidectomy, and has been often seen after the administration of large amounts of thyroid extract. There seems also to be a close connection between diabetes and gout, arteriosclerosis, and obesity.

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[1] See for details on this subject, *Opie:* Diseases of the Pancreas, Philadelphia, 1903.

A peculiar form of glycosuria has been described as occurring in vagrants without the accompaniment of any of the inciting conditions mentioned above.

## DIABETES

The amount of glucose which the urine may contain in diabetes is enormous. It may rise to over 10 per cent., and the total excretion may approach 1,500 grams in the twenty-four hours.

The urine is usually pale, transparent, and acid, and is passed in large quantities, often as high as five, occasionally as high as ten or even twenty liters. The odor of acetone is often strongly marked. The amount of sugar varies with the time of day and the time that food is ingested. The minimum amount is during the night hours; during the day there are, as a rule, two maxima, one about noon and the other toward six. These variations are dependent on the time of meals. In severe cases the sugar may be excreted continuously and even more abundantly at night. It is in the light cases that the greatest variations occur; during the night the sugar may entirely disappear. Other light cases will show a small amount of sugar during a period of a few weeks, and then for some time the urine may be entirely free from any glucose.

The sugar sometimes alternates with the albumin which is often present in the urine of diabetes; and, finally, one of the most powerful influences in producing fluctuations in the sugar content of the urine in diabetes is the condition of the nervous system. A sudden nervous shock will sometimes cause sugar to appear in the urine of a diabetic from which it has long been absent owing to careful regulation of the diet.

**The clinical types in diabetes are three:** the mild forms, the severe, and the intermediate forms.

1. In the mild forms the urine can be freed from sugar by limiting the intake of carbohydrates. It may be necessary only to stop taking more than 100 grams of carbohydrate, or it may be necessary to limit the ingestion entirely. At times the condition

of the patient improves and the glucose does not reappear even after considerable amounts of sugar are eaten.

2. In the severe forms the urine can not be freed from sugar by withdrawal of the carbohydrates; in some it is even impossible to free the urine of sugar by reducing the proteid intake; in others the urine becomes sugar-free when the proteids are reduced to a certain point, but just as soon as the amount of proteid is again increased the sugar split off from it begins to appear in the urine.

It is probable that some of these severe cases obtain a portion of their sugar from the metabolism of the fats. Lusk,[1] however, believes that the sugar is derived entirely from the proteid, possibly by a synthetic process, and points out that both in phlorizin dogs and in some severe and rapidly fatal cases of human diabetes it is possible to obtain a constant ratio between the dextrose excreted and the nitrogen in the urine; that is, about 3.6 grams of glucose are excreted for every gram of nitrogen taken in, this representing the maximum output of sugar from proteid and complete inability of the body to oxidize carbohydrates. This condition has been found by Lusk in a rapidly fatal case of diabetes, the urine of which contained no albumin, but little ammonia, no $\beta$-oxybutyric acid, and only a small quantity of acetone. The approximation of the sugar-nitrogen ratio to 3.6, therefore, should be regarded as a sign of exceedingly bad prognosis as regards the life of the patient.

3. The intermediate forms are those in which the sugar cannot be removed from the urine by simply removing the carbohydrate from the food, but in which it is possible to remove it by limiting somewhat the proteid food. As a rule those forms can be considered as intermediate in which the nitrogen excretion in the urine must be kept below 18 grams, and may be kept above 10 grams. In children the upper limit is 13 grams, the lower limit 7 grams of nitrogen. The severe forms are those in which the nitrogen in the urine must be kept below 10 grams in adults and 7 grams in children. It will be seen, under the discussion of the subject of acetone, that the amount of sugar in the urine is not the only point of diagnostic and prognostic value.

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[1] *Mandel* and *Lusk:* Proceedings of the Society for Experimental Biology and Medicine, 1904, p. 36: *Stiles* and *Lusk:* Journal of Physiology, vol. x, 1904, p. 67.

## QUALITATIVE TESTS FOR GLUCOSE

**Trommer's Test.**—Ten c.c. of the urine are rendered alkaline with a few drops of 10 per cent. sodium or potassium hydrate. Dilute copper sulphate solution is then added until the precipitate of copper oxide is no longer dissolved. If glucose or lævulose is present a deep blue color is produced. On warming the mixture a reddish copper oxide separates when sugar is present.

In order to avoid serious errors, the diagnosis of the presence of glucose should only be made from this reaction when the red copper oxide separates from the solution before the boiling point is reached. When traces of sugar are present this reaction does not take place until after considerable boiling, but under these circumstances pentoses and some of the conjugate compounds of glycuronic acid may cause a separation of the oxide copper.

It has been pointed out [1] that if large quantities of alkali are added the red oxide is precipitated by the action of sugar, while the yellow product is due to the action of creatinin. This substance can be changed into creatin by excess of alkali and then does not exert this reducing reaction. It is therefore important to have a considerable excess of the sodium hydrate. On the other hand, if too much alkali is added only a grayish color is produced when the mixture is heated.

This test is not a reaction for sugar. It is merely a test of the reducing power of the urine, and therefore may be given by any reducing substance, such as uric acid, creatinin, or chloroform. The last substance usually produces a darker precipitate than sugar.

**Fehling's Test.**—Equal quantities of Fehling's alkaline and copper sulphate solution are mixed and heated in a test tube to the boiling point. A small quantity of the urine to be tested is then added and the mixture boiled. If no reduction takes place a few more c.c. of the urine are added and the boiling repeated. If the fluid turns yellow and then a copper red color, the presence of a *reducing substance* is certain. Whether this reducing substance is glucose or not depends upon other tests. Only the red copper suboxide is of diagnostic value; a yellow or a greenish precipitate is often seen in normal urines. The boiling should not be too prolonged, as some of the conjugate glycuronates may be split up and then give a reduction.

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[1] *Neumayer:* Deut. Arch. f. klin. Med., Bd. lxxvi, 1900, p. 197.

**Phenylhydrazin Test.**—*v. Jaksch method.* The urine is diluted with an equal bulk of water, or more if the quantity of glucose is large, and about 1 gm. of dry phenylhydrazin hydrochloride and 2 gms. of sodium acetate are added to about 10 c.c. of the urine. The fluid is heated for twenty minutes on a water bath and then placed in cold water for a short time to facilitate the separation of the glucosazon crystals.

A neat and easily extemporized water bath for a test tube can be made by filling an Erlenmeyer flask of about 250 c.c. capacity with a little water and standing the tube upright in the narrow neck of the flask. Very little evaporation takes place when the flask is heated.

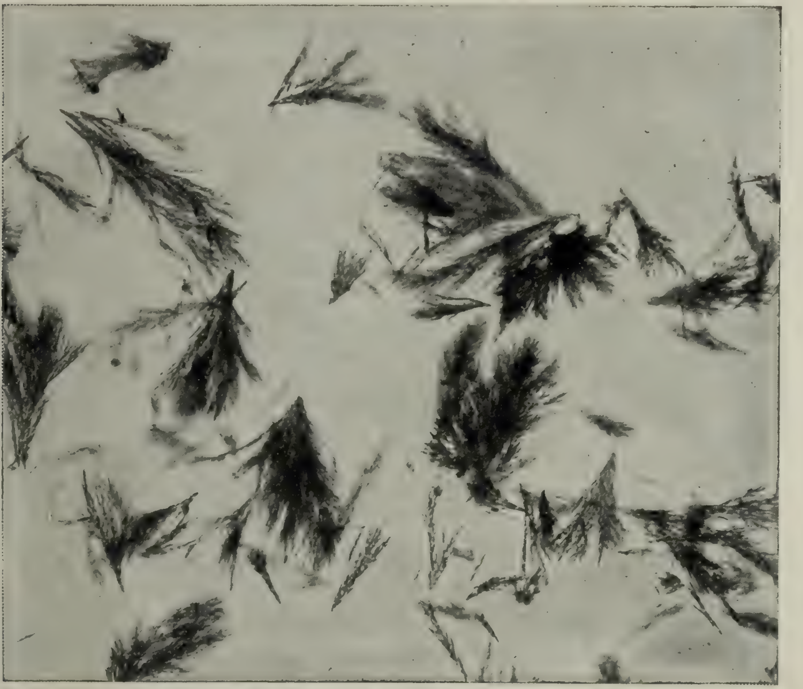

FIG. 142.—CRYSTALS OF PHENYLGLUCOSAZON.

If the quantity of sugar is rather large, an abundant yellow precipitate of the glucosazon appears before the urine is cooled. If, on the other hand, the quantity of sugar is very small, only enough crystals will be formed to give a slight deposit at the bottom of the test tube after the contents have been thoroughly cooled. The crystals are long, fine yellow needles arranged in sheaves and spherical masses. Crystals only are characteristic; dark, highly refractile globules of a deep yellow color are not of any diagnostic value.

*Kowarsky's method* is more convenient and simpler. Five drops of phenylhydrazin are mixed in a test tube with 10 drops of glacial acetic acid and 1 c.c. of a saturated solution of sodium chloride. A curdy mass is formed of phenylhydrazin hydrochloride and sodium acetate. Then add 2 to 3 c.c. of the urine to be tested and heat for at least two minutes over a flame. If over 0.5 per cent. of glucose is present an immediate formation of the glucosazon is observed. If smaller amounts are present the test tube should be allowed to cool for a few minutes and the crystals which have collected at the bottom removed with a pipette and examined under the microscope with a one-fifth objective.

If only a few single crystals are seen, and if the other tests for sugar have been negative, especially if the urine does not ferment with yeast, the urine should not be considered as possessing an abnormal amount of sugar, for normal urine will occasionally furnish a few needles of an osazon which may be derived from the sugars normally present. In the Kowarsky method so small a quantity of urine is used that this complication is not likely to occur, and any crystals which are found may be looked upon as an evidence of the presence of sugar in pathological amounts. The small quantity of isomaltose present in a normal urine will not give a maltosazon, for this compound is soluble in four parts of water. The glycuronates and lactose do not furnish crystals under ordinary conditions, so that, when properly carried out, this test is one of the best for the identification of glucose in the urine. Lævulose gives an osazon identical with phenylglucosazon, while the crystals of the phenylmaltosazon can be distinguished microscopically by the fact that the yellow needles are much broader than those of the phenylglucosazon.

**Nylander's Test.**—The reagent is composed of 2 grams of bismuth subnitrate, 4 grams Rochelle salt, 10 grams sodium hydrate in 100 c.c. of water. It keeps well in dark bottles.

Albumin must be removed from the urine by boiling and filtration before testing. The urine is placed in a small test tube and one-tenth volume of the reagent added and the whole slowly heated. If over 0.1 per cent. of glucose is present the fluid turns dark brown and then black.

A brown color may appear, which is not due to glucose, and which does not finally change to black, but occurs when the urine contains the products excreted after the administration of rhubarb, senna, antipyrin, camphor, salicylic acid, chloroform, saccharin, chloral hydrate, and turpentine. The reducing power of some of these substances is due to the capacity which they possess to form conjugate compounds with glycuronic acid. If, however, the test is carefully carried out and only a dense black precipitate be considered as positive, it becomes one of the best and most useful tests for sugar for general use by the practitioner.

## QUANTITATIVE TESTS FOR GLUCOSE

**Fehling's Method.**[1]—This test depends upon the power of glucose to reduce copper oxide in an alkaline solution. The solution, which may be used for either quantitative or qualitative tests, is kept in two separate parts made up as follows:

*a.* Exactly 69.278 grams of chemically pure crystals of copper sulphate are dissolved in about 600 c.c. of warm distilled water, and after the solution has cooled to the temperature for which the measuring flask is corrected the whole is made up to 1,000 c.c.

*b.* About 346 grams of sodium and potassium tartrate (Rochelle salt) and 100 grams of sodium hydrate, "purified by alcohol," are dissolved in water and the whole made up to 1,000 c.c. after the fluid has cooled. The solutions are to be mixed only at the time of using.

To test quantitatively for sugar in the urine, 5 c.c. of each solution is carefully measured off and 40 c.c. of water is added. The fluid is then either boiled in a porcelain dish or in a beaker or Erlenmeyer flask, and while hot the urine to be tested is run in from a burette about 1 c.c. at a time. The precipitated cuprous oxide usually settles quickly to the bottom, and the supernatant fluid can be seen to grow less blue with each addition of the urine.

If the oxide does not settle rapidly and the color of the fluid can not be seen, remove a drop of the fluid from the beaker and let it fall upon one side of a double sheet of filter paper of good quality. As soon as the drop has moistened the second sheet, remove it and touch the moistened spot with a little dilute acetic acid and a drop of potassium ferrocyanide. A brown spot of copper ferrocyanide shows that all the copper is not yet reduced. The reason for the use of the two sheets of paper is to prevent the copper oxide from being dissolved when the acid is added, as would be the case if only one sheet were used. All the oxide is caught in the pores of the first paper and removed, the test being carried out solely on the filtrate on the second paper. A drop of the solution can thus be removed from time to time and the exact point at which all the copper is reduced from the solution determined. A very faint brown may be neglected, as it is due to the copper oxide held in solution by the ammonia set free in the mixture by the action of the alkali.

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[1] *Fehling:* Archiv f. physiol. Heilk., 1848, p. 64.

The reduction of the Fehling's solution is much more accurate when the sugar solution is added as rapidly as possible, so that the first titration must be considered as merely a preliminary test to roughly estimate the amount of sugar present. In the second titration the urine should be added at once to within half a cubic centimeter of the result of the first titration, and then the final adjustment by drops can be made in a few moments without giving the solution any chance to obtain oxygen from the air. If considerable accuracy is required a third titration should be made, using 20 c.c. of the Fehling's solution.

*Precautions.*—Albumin in traces does not interfere with the reduction of Fehling's solution, but if present in amounts approximating 0.2 per cent. it delays the precipitation of the copper oxide from the solution and must first be removed from the urine by the addition of acid and boiling. The urine should contain less than 1 per cent. of sugar. If there is more sugar present the urine must be carefully diluted and the dilution allowed for in calculating the result.

*Calculation of the Result.*—Fehling's solution is so made up that the 10 c.c. used are reduced by, and therefore correspond to, 0.05 gram of glucose. If, then, it has required 8 c.c. of urine to reduce all the copper, evidently this 8 c.c. of urine must contain 0.05 gram of glucose. One c.c. of urine, therefore, will contain one-eighth of this amount, or 0.0062 gram of glucose. If, then, 1 c.c. of urine contains 0.0062 gram of glucose, 100 c.c. will contain 0.62 gram of glucose, or, in other words, the urine will contain 0.62 per cent. of sugar.

**Pavy Method.**—If the urine, as is often the case, does not reduce the oxide of copper to the red cuprous oxide immediately, but yields a yellowish-green fluid from which no oxide separates out, it is impossible to decide upon the exact point at which all the copper is reduced, and it is necessary to modify the method so as to allow of a determination being made. This is best accomplished, as suggested by Pavy,[1] by the addition of 30 c.c. of strong ammonia to the mixed solution. As the reduction in an ammoniacal solution is different from that in one which contains only fixed alkali, it is necessary to use 12 c.c. of the mixture instead of 10 c.c. That is, we measure off 6 c.c. of each of the alkaline and copper solutions and add 30 c.c. of ammonia and also 60 c.c. of water.

If to this diluted ammoniacal solution we add 10 c.c. of a 50 per cent. solution of sodium hydrate, the reduction takes place according to the original formula, i. e., 10 c.c. = 0.05 gram of glucose.[2] The method of preference for accurate sugar determinations is to make up the copper solution and then standardize it against a weighed amount of pure glucose.

The reduced copper in this solution is not precipitated, but is held in solution by the ammonia, and the color simply grows paler as the reduction takes place, and finally when it is complete becomes suddenly decolorized. The decolorization is never quite complete, for there is always a yellow tint to the solution from the action of the alkali on the sugar present. The change, however, is so marked that the end reaction is very easily determined.

If the decolorized solution is allowed to stand for a few minutes it rapidly regains its original blue tint, so that the whole titration must be carried out as quickly as possible, and is best done in a narrow-necked flask.

If a large number of determinations are to be made by this method, it is well to have the neck of the flask fitted with a cork through which the tip of the burette passes, and another tube to convey the fumes from the ammonia into a bottle containing fragments of pumice-stone wet with strong sulphuric acid to absorb the ammonia as it is volatilized. The calculation is the same as with the Fehling's; that is, the number of cubic centimeters of urine used corresponds to 0.05 gram of glucose.

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[1] *Pavy:* Zeit. f. anal. Chem., Bd. xix, 1880, p. 98. Also Chem. News, vol. xxxix, 1879, p. 77.

[2] *Pavy:* Jour. of Chem. Soc., vol. xxxviii, 1880, p. 512.

As has been previously stated, the normal reducing power of the urine may be from at least 0.08 to 0.6 per cent., so that the results of sugar titrations are always too high by that amount; and as the error of the titration methods is also at least 0.1 per cent., it can easily be seen that the process is rather clinical than scientific in its accuracy.

The error is less, however, if the urine is strongly diluted, for then the uric acid, creatinin, and other substances interfere to a much less extent. Greater accuracy can be obtained by titrating the urine before and after fermentation, the difference between the results giving the glucose. It is occasionally of advantage to shake up the urine with a few crystals of lead acetate, which removes a portion of the substances interfering with the process of reduction.

**Rudisch Method.**—Another solution which has some advantages over that of Pavy has been suggested by Rudisch.[1]

This solution contains

| | | |
|---|---|---|
| Copper sulphate, crystallized, | 4.17 | grams. |
| Sodium sulphite, crystallized, | 50.00 | " |
| Sodium carbonate, crystallized, | 80.00 | " |

The salts are dissolved in 10 per cent. ammonia water and made up to 500 c.c. One c.c. of this solution is decolorized by one milligram of glucose.

Ten c.c. of the standard solution diluted with 40 c.c. of water are put in an Erlenmeyer flask and heated. The flask should be arranged with a tube leading into sulphuric acid in order to avoid annoying ammonia fumes. The urine, which should preferably be decolorized by filtration through animal charcoal, is allowed to drop into the hot copper solution until the color disappears.

If large quantities of sugar are present the urine should be correspondingly diluted.

When very exact results are required the sugar normally present in the urine should be determined by titrating equal amounts of the specimen before and after fermentation. The fermentation should be carried out at a temperature of about 37° C. for eighteen hours. Ordinary yeast may be used, or, if preferred, a pure culture of Saccharomyces apiculatus.

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[1] Jacobi Festschrift, New York, 1900, p. 354.

**Determination of Glucose by Means of the Polariscope.**—The type of instrument ordinarily employed for clinical work is the so-called half shadow apparatus. It consists essentially of a Nichols' prism *P*, which acts as a polarizer, a tube *R* to carry the urine to be tested, and a second Nichols' prism or analyzer *A* by which the amount of rotation caused by the fluid is measured. The zero of the instrument is fixed at the point at which the two lateral halves of the field of the eye-piece are equally illuminated.

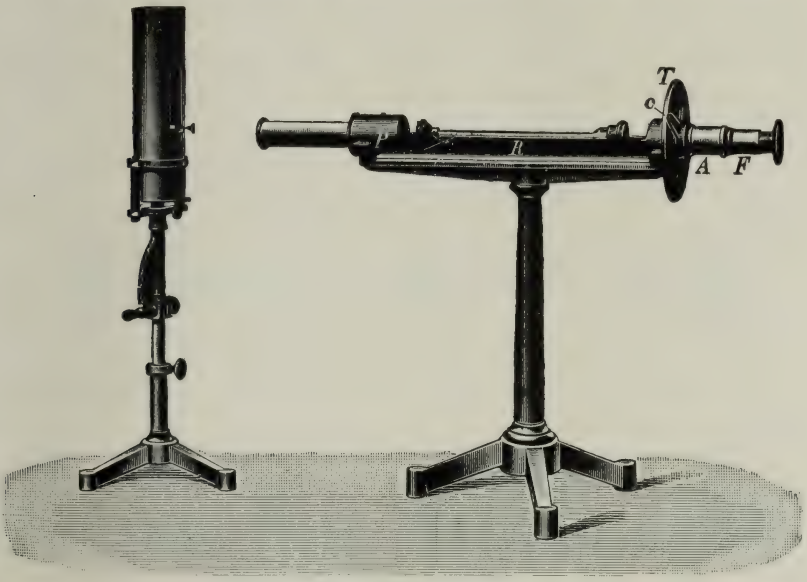

Fig. 143.—Polariscope.

In this type of apparatus it is necessary to employ monochromatic light, the most convenient for this purpose being that produced by the vaporization of a sodium salt in the Bunsen flame.

The position of the zero on the scale should be determined before using the instrument, as this may be altered by moving the apparatus. The observation tube is then filled with the urine which has been acidified with acetic acid and precipitated with a small amount of solid lead acetate and filtered. The tube, if not perfectly dry, should be washed out with distilled water. The latter should be removed by pouring a small quantity of the filtered urine into the tube and then emptying the tube again. This should be repeated at least once, for if water be left in the tube, currents are formed between the water and the denser urine and the sharpness of the image of the two halves of the fluid is greatly interfered with. One end of the tube is closed with a cap, the other end is filled a little more than even full and the glass cover slid on sideways in order to avoid leaving a bubble of air in the tube. The brass cap is then screwed on with moderate firmness. Excessive pressure will either crack the polished end of the observation tube or will cause optical changes in the glass which interfere with the readings.

Recent forms of tubes have caps which slide on instead of screwing on, and are to be preferred. They also have a space for receiving any air bubbles imprisoned in the upper end of the tube, thus allowing for expansion of the fluid (Fig. 144, *a*).

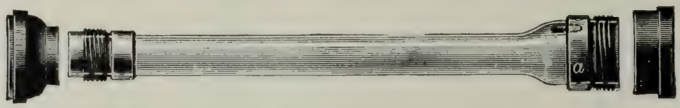

Fig. 144.—Tube to Hold Fluids for Polariscope.

The light is arranged at a proper distance from the end of the instrument and some fused sodium chloride is placed in the platinum cup. A mixture which is somewhat preferable to sodium chloride is made up by fusing equal parts of sodium phosphate and sodium chloride and then pulverizing the fused mass. The mixture should be kept in closely stoppered bottles in order to prevent the absorption of water.

After the tube is placed in position the eye-piece is drawn out until the two divisions of the field are separated by a sharp line. For this purpose it is necessary to rotate the handle through 20 or 30 degrees so as to get a strong contrast between the two fields. The handle is then turned until the vernier approaches zero and the brightness of the two halves of the field is rendered as nearly as possible equal. The vernier is then read by means of a hand lens and the observation noted. This should be repeated three or four times and an arithmetical mean taken of the results.

The observation tube must be cleaned immediately after use, and care should be taken that the caps are not left tightly screwed down, as otherwise the rubber adheres to the glass and renders it difficult to remove the plate-glass disks.

*Calculation of the Results.*—The specific rotation of glucose is $[\alpha]_D^{20} = +52.74°$, but for practical purposes in urine it may be assumed as $+53°$.

In order to compute the amount of sugar in per cent. the following formula may be used:

$$g = \frac{a \times 100}{53 \times l}$$

g = the amount of glucose in grams in 100 c.c. of the solution.

a = the angle of rotation.

l = the length of the tube used in making the observations.[1]

The length of the tube for clinical polariscopes is so arranged that the readings can be made directly in percentages. Two tubes are supplied, a short one measuring 94.7 millimeters in length, each degree of rotation corresponding to 2 per cent. of *glucose;* while the other is twice this length, or 189.4 millimeters, each degree of deviation corresponding to 1 per cent. of *glucose.* The short tube gives less accurate results but can be used without decolorizing the urine, while with the longer tube lead acetate must always be added to the urine in order to clear and decolorize the fluid.

The vernier of the apparatus permits readings to one-tenth of a degree, but a number of readings should be made and the average taken. Small amounts of lævorotatory glycuronates lower the rotation, so that in urine the accuracy of the instrument is scarcely higher than a fifth of 1 per cent.

**The Fermentation Test.** *Einhorn Saccharometer.*—The most convenient method of applying this test is to use one of the small Einhorn fermentation tubes, which are graduated to read the per cent. of sugar from the bulk of $CO_2$ evolved. The urine must contain less than 1 per cent. of glucose, and for practical purposes a urine of a specific gravity of 1.020 should be diluted with an equal bulk of water, that of 1.025 with two parts, etc. Allowance must be made for the dilution by multiplying the final results with the corresponding factors.

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[1] For details of the theory and practice of the polariscope, see *Landolt:* Optische Drehungsvermögen, Braunschweig, 1898.

The urine, after the proper dilution has been carried out, is to be gently shaken up with a fragment of fresh cake yeast about the size of a split pea. The shaking should not be so violent as to cause any foaming, for the air so distributed through the fluid will slowly collect at the top of the tube and may be taken for gas produced by fermentation. If the urine is alkaline it should be rendered faintly acid with hydric acetate. The U-tube is then carefully filled with this mixture of yeast and urine, avoiding all air bubbles at the upper end. An exactly similar preparation is then made with a urine which is known to be normal, and the two tubes set aside at room temperature.

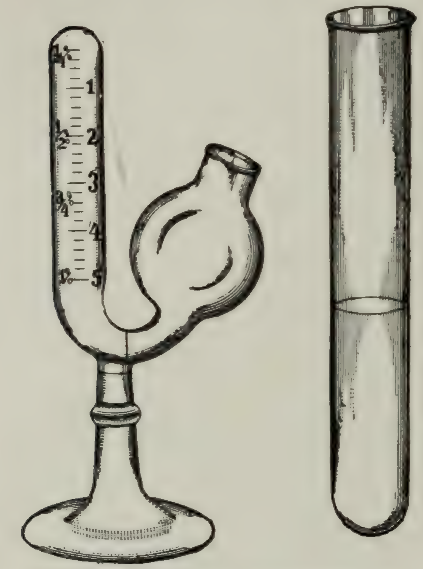

FIG. 145.—EINHORN SACCHAROMETER.

In a few hours, if the sugar is abundant, bubbles of gas will begin to collect at the top of the tube, and at the end of fifteen to eighteen hours the fermentation will be complete and the amount of sugar may be read off. The reason for the control tube is that certain varieties of yeast undergo what is called self-fermentation; that is, there is enough gas given off during the growth of the yeast, and possibly also from a slight amount of sugar which may be present, to form a considerable volume at the top of the tube. This gas which collects in the control tube should be subtracted from the reading of the tube of the diabetic urine.

The fermentation method is the most convenient and satisfactory method for the practitioner, and it is also the most certain test for the presence of glucose in the urine. For scientific purposes it is best to use, instead of ordinary yeast, a pure culture of the yeast plant Saccharomyces apiculatus. Maltose and lævulose, if present in the urine, are fermented by the yeast; lactose is not, at least during the first twenty-four hours. Glycuronic acid is not fermented.

*Lohnstein Saccharometer.*—A more accurate fermentation apparatus which can be used for urines containing large quantities of sugar has been devised by Lohnstein.[1] The errors which arise from the use of the Einhorn fermentation tube at widely differing temperatures, etc., are avoided by the proper graduation of the scale for two temperatures, and by separating the urine to be fermented from the atmosphere by mercury. (See Fig. 146.)

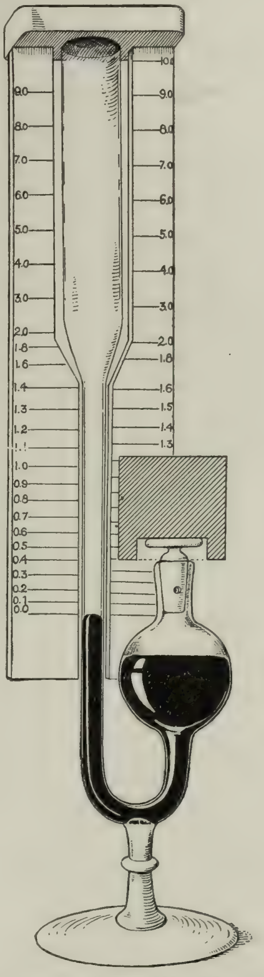

FIG. 146.—LOHNSTEIN'S SACCHAROMETER FOR UNDILUTED URINE.

The apparatus consists of a bulb and a tall U-tube connected with it, from the long arm of which hangs a movable scale of temperatures and percentages of sugar. The bulb is closed at the top by a ground-glass stopper which has an opening which corresponds with the opening in the neck of the bulb, so that the air space which remains above the mercury with which the bulb has been partly filled can be made to communicate or not with the outside air. When fermentation takes place the pressure on this stopper is very considerable, so that a weight is provided to place on the top of the stopper and hold it in position. The amount of mercury (12 c.c.) which should be used is furnished with the apparatus. To make a sugar determination one-half c.c. of the urine to be tested is taken up in a small syringe furnished with the apparatus and injected on the surface of the quicksilver. The syringe is then washed out and filled for about ten divisions with a thick paste made out of ordinary compressed yeast diluted two or three times its volume with water. This paste is injected into the bulb on top of the mercury and mixed with urine. The stopper is then carefully greased with a mixture of equal parts of yellow wax and vaselin, turned so that the two apertures correspond, and by gently tipping the apparatus the column of mercury in the straight arm of the tube is made to coincide with the zero of the scale. When this occurs the stopper is readjusted so that the holes no longer correspond, the apparatus is brought back to the vertical, the weight is placed on top of the stopper to prevent leakage, and the whole apparatus is placed in the incubator. In six hours the fermentation is complete. The apparatus is allowed to stand in the air for a few minutes to regain room temperature and the scale is read off. Or the mercury may be adjusted after the apparatus has been warmed in the incubator and the readings may be taken at incubator temperature, as one side of the scale is graduated to correspond with 35° C. If an incubator is not accessible the fermentation can be allowed to go on at room temperature, but this requires considerable time, from eighteen to twenty-four hours, to complete the fermentation of the last portions of the sugar. Or the apparatus can be stood in a vessel of water and this water heated by either a small gas burner or a night light candle, the distance from the flame being so regulated that the temperature remains about 32° or 33° C. In this way fermentation can be completed in about six hours.

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[1] Münch. med. Woch., 1899, p. 1671.

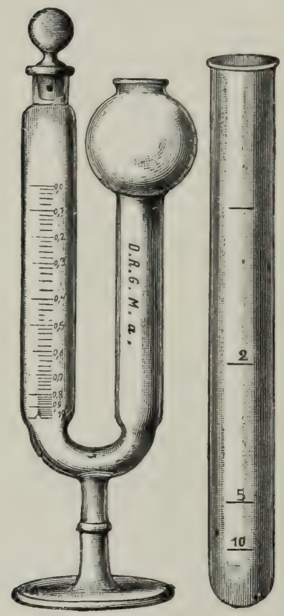

Fig. 147.—Lohnstein's Saccharometer for Diluted Urine.

After the determination is completed the stopper is turned slowly until the holes correspond, while the mercury returns to its former level. The stopper is then taken out and the bulb cleaned with a little absorbent cotton. The space over the mercury is then washed out several times with fresh water, and this water is removed by a fresh piece of cotton. After long use the mercury becomes contaminated, and it should be turned out, filtered through a pinhole in a piece of filter paper or squeezed through chamois leather, and the glass part of the apparatus cleaned out with hot sodium carbonate solution. The results obtained with this fermentation apparatus correspond very closely with those of the titration and polariscopic methods. It furnishes, therefore, a very convenient and simple means for the determination of sugar by the practitioner.

Another form for the apparatus using diluted urine is shown in Fig. 147.

### LÆVULOSURIA

Lævulose has been found in the urine in a few cases, of which the most authentic are those of May,[1] Rosin and Laband,[2] and Schlesinger.[3] Other reported cases by Zimmer,[4] Seegen,[5] Külz, and Marie and Robinson,[6] are less certain, owing to the imperfect identification of the sugar excreted. Of the observed cases few gave symptoms referable to diabetes or excreted very large quantities of lævulose, except Zimmer's patient, where the amount of the sugar present reached 10 per cent.

The case of Laband showed a lævorotation of 1.2°, which is equivalent to about 0.9 per cent. of lævulose. After the ingestion of 100 grams of lævulose the amount present in the urine was *diminished.* One hundred grams of dextrose also did not produce an alimentary glycosuria. The diabetic symptoms gradually disappeared. Lævulose was found in the blood. In the other cases the amount of sugar was moderate, and in all a prompt reduction in the amount in the urine was obtained by withdrawing carbohydrates from the diet. The administration of lævulose and saccharose in about 100 gram doses in Schlesinger's case caused a great increase in lævulose excretion, and this fact is of especial interest, because it has long been known that in true dextrose diabetes large quantities of lævulose can be assimilated without increasing the sugar excretion in the urine. The injection of phloridzin caused a large excretion of dextrose but no increase in lævulose.

The condition is evidently a transitory one, as it can be easily controlled by proper regulation of the carbohydrate intake, and should be sharply separated from glycosuria, for none of the cases were observed to go on to true diabetes with the excretion of dextrose, so that it seems probable that cases of lævulosuria are to be considered as due to some defect in the normal metabolism of that sugar to glycogen in the liver, and therefore an interference with its final use as a source of energy for the tissues. It has been also suggested that this sugar may be formed in the body and the excess excreted, an idea which has also been developed to explain the appearance of pentose in the urine. Rosin and Laband have also shown that many diabetic patients excrete small quantities of lævulose in the urine, a fact of scientific interest only. The blood of these patients also contains lævulose. Neuberg and Strauss[1] have also shown that lævulose is present in small amounts in serous exudates.

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[1] Deut. Arch. f. klin. Med., Bd. lvii, 1902, p. 279.

[2] Zeit. f. klin. Med., Bd. xlvii, 1902, p. 182. See also Lépine et Boulud. Rev. d. Méd., 1904, p. 185.

[3] Arch. f. exp. Path. u. Pharm., Bd. xv, 1903, p. 273.

[4] Prag. med. Woch., 1876, p. 330.

[5] Cent. f. d. med. Wissen., 1884, p. 753.

[6] Semaine Méd., 1897, p. 250.

The administration to a fasting person of a solution of 100 grams of lævulose or honey in 500 c.c. of water incites a lævulosuria in about 15 per cent. of the cases examined. It has been shown[2] that in about 85 per cent. of persons suffering from diseases of the liver the administration of a similar amount of lævulose will incite a marked lævulosuria. A considerable variety of hepatic diseases show alimentary lævulosuria. In cirrhosis it frequently appears; in carcinoma of the liver it has also been observed; and recently it has been found that alimentary lævulosuria occurs in icterus due to syphilitic changes in the liver.[3] All cases of spontaneous lævulosuria should be examined for lesions of the liver.[4]

In order to determine the presence of lævulose the urine should be fermentable, rotate to the left, reduce Fehling's solution and give Seliwanoff's reaction.

**Tests for Lævulose.**—Inasmuch as lævulose reduces Fehling's solution, ferments with yeast, and forms an osazon with a melting point of 205° C., it can not be distinguished from glucose by these methods. The slight lævorotation which is produced by the small quantity of this sugar usually present in the urine may easily be confused with that due to glycuronic acid. There are, however, two reactions which can be used to identify lævulose.

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[1] Zeit. f. phys. Chemie, Bd. xxxvi, 1902, p. 225.

[2] *Strauss:* Deut. med. Woch., 1901, pp. 757 and 786; *Bruining:* Berl. klin. Woch., 1902, p. 587; *Steinhaus:* Deut. Arch. f. klin. Med., 1902, Bd. lxxiv, p. 537; *Chajes:* Deut. med. Woch., 1904, p. 696.

[3] *Šamberger:* Arch. f. Derm. u. Syph., Bd. lxvii, 1903, p. 94.

[4] See for an interesting case of alimentary lævulosuria, *Schwartz:* Deut. Arch. f. klin. Med., Bd. lxxvi, 1903, p. 286.

**Seliwanoff's Reaction.**[1]—Ten c.c. of the urine are heated with a small amount of resorcin and 2 c.c. of dilute hydrochloric acid. If lævulose is present a brilliant red color will appear. It has been generally assumed that this reaction was characteristic of the ketose group of sugars, but it has recently been found that the same reaction may be given by some of the polysaccharids, namely, cane sugar, raffinose, and inulin, and even by cellulose. Where these substances can be excluded the reaction may be regarded as an evidence of the presence of either of the two ketose sugars, lævulose and sorbinose, and must remain the only characteristic reaction which can be ordinarily applied by the practitioner.

The Seliwanoff reaction has recently been improved by Rosin.[2] After heating the fluid to be tested with an equal bulk of hydrochloric acid and a few granules of resorcin, if the characteristic red color be obtained the fluid is to be cooled and neutralized with sodium carbonate. The orange-colored, alkaline fluid is now shaken out with amyl alcohol, which extracts a red coloring matter which shows a slight shade of yellow and a green fluorescence.

On the addition of a few drops of absolute ethyl alcohol the color becomes pure rose red. Dilute solutions show a single band of the green extending from the E line to b. In more concentrated solutions this band becomes darker and extends somewhat over the two lines mentioned, while a second very faint band appears in the blue near the line F. The addition of alcohol increases the sharpness of the band in the green. The reaction and spectrum is given only by the ketoses, especially lævulose and sorbose, and also by hydrochloride of glucosamin, but the reaction in this case is very faint.[3] The absorption spectrum, however, permits a differentiation between the reaction produced by the two ketoses and the reddish color which is produced by other substances with hydrochloric acid and resorcin.

The Seliwanoff reaction, lævorotation and the action of this sugar on Fehling's solution and its fermentability by yeast are the only tests which come within the scope of a clinical laboratory.

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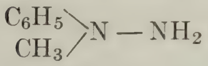
[1] Chem. Berichte, Bd. xx, 1887, p. 181.

[2] Zeit. f. phys. Chemie, Bd. xxxviii, 1903, p. 555.

[3] *Fr. Müller:* Zeit. f. Biologie, Bd. xlii, 1901, p. 511.

**Neuberg**[1] has recently shown, however, that lævulose forms a characteristic osazon with asymmetrical methylphenylhydrazin  $\left.\begin{matrix}C_6H_5\\CH_3\end{matrix}\right\rangle N-NH_2$ even in the presence of other sugars, and that this is the best means of identifying lævulose.

The method is as follows : The urine, or other fluid which is to be investigated, is rendered acid with a few drops of acetic acid as soon as possible after it has been obtained. The fluid is brought to the boiling point and the albumin which separates filtered off. The clear filtrate, which must be distinctly acid, is then evaporated in a vacuum to a thin sirup, at a temperature not above 40° C. A few fragments of litmus paper are left in the fluid in order to see that it remains acid during the entire course of the evaporation. The residue is then extracted with 98 per cent. alcohol by heating over a water bath, the amount of alcohol used being half of the quantity of the original fluid taken. In about five minutes the major portion of the lævulose is extracted and the fluid is filtered after it is cold. If the residue which remains after extraction with alcohol shows a considerable reducing power to Fehling's solution after dissolving in water, it should be re-extracted with the same amount of alcohol. The united alcoholic extracts are filtered if necessary to remove any flocculent precipitate and then decolorized with bone charcoal.

A measured quantity is removed and the amount of reducing substance determined by titration with Fehling's solution. The quantity of substance thus determined is considered as lævulose, and the proper amount of methylphenylhydrazin is added to the alcoholic solution after the latter has been evaporated to about 30 c.c. The amount of methylphenylhydrazin is three molecules for one molecule of sugar—that is, for each gram of sugar, two grams of the reagent should be added. The mixture is allowed to stand in the cold for several hours, and, in case a precipitate has formed, is filtered. The filtrate is mixed with an amount of 50 per cent. acetic acid equal to the amount of methylphenylhydrazin which has been used, and enough alcohol is added to produce a clear solution. The mixture is then heated for from three to five minutes on a water bath, or better, in an incubator at 40° C., for twenty-four hours. If large amounts of lævulose are present the methylphenylosazon will separate out in crystalline form, and especially after the addition of a few drops of water. If small quantities only are present the addition of water causes separation of the osazon in the form of an oily fluid, which will crystallize if rubbed with a glass rod, or after inoculation with some crystals of lævulose-methylphenylosazon. The same result can be obtained more rapidly by very strong cooling as follows:

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[1] *Neuberg* u. *Strauss:* Zeit. f. phys. Chemie, Bd. xxxvi, 1902, p. 225.

The oily methylphenylosazon is separated from the mother liquor and washed several times by decantation with cold water, and finally dried in a vacuum over concentrated sulphuric acid. The resulting mass is dissolved in alcohol, filtered, and placed in a cooling mixture of ether and solid carbonic acid. Crystallization begins almost immediately. The crystals are separated from the fluid and washed with absolute alcohol which has been chilled in the same cooling mixture.

The crude product can be purified by dissolving in hot water with the addition of a small amount of pyridin. A small amount of bone charcoal is added to the fluid; it is filtered, and evaporated to a small bulk, during which procedure the osazon separates in delicate yellow needles of a melting point of 158° to 160° C. The preparations recrystallized from alcohol show a more reddish color and generally a lower melting point, about 153° C. Two decigrams of the methylphenyl-lævulosazon dissolved in a mixture of 4 c.c. of pyridin and 6 c.c. of absolute alcohol show a dextrorotation of 1° 40′ in a 10 cm. tube. The yield of the osazon is about 80 per cent. of the amount of sugar present in pure solutions, and about half that amount in urine and exudates.

## LACTOSURIA

Lactose is found in small quantities, usually less than 1 per cent., in the urine of many women after childbirth. The older idea that lactose appeared in the urine of pregnant women has been shown to be incorrect. The sugar appears in about 20 per cent. of nursing women, but, on the other hand, in about 80 per cent. of those who do not nurse their children. The sugar appears most frequently in the first few days after delivery, and may continue in the urine for a number of months. It is usually most abundant[1] about the fourth or fifth month after delivery, and averages about 0.35 per cent., the amount depending upon the condition of the breasts and the quantity and quality of the milk. In women who nurse their children there is usually no lactose present in the urine, but if for any reason nursing is interrupted very considerable quantities of lactose appear, and as this may occur during any part of the post-partum period, it is of importance to be able to distinguish lactose from glucose in order to avoid an incorrect diagnosis of diabetes based upon the discovery of a reducing sugar in the urine. The reason for the appearance of lactose in the urine is that it is absorbed into the blood directly from the glands of the breast and immediately excreted unchanged by the kidneys. The addition of considerable quantities of glucose to the food may increase the amount of lactose in the blood, showing that some of the lactose must be used by the tissues, and that the additional glucose prevents the oxidation of the lactose in the blood or its absorption.

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[1] *MacCann:* Lancet, 1897, p. 1174; also *Pavy:* ibid., p. 1075.

In order to differentiate lactose from glucose it is necessary to determine the presence of a reducing substance in the urine and then to carry out a fermentation test, preferably with a pure culture of yeast. If only the ordinary compressed yeast is available, the fermentation must be interrupted at the end of a few hours, because the bacteria present are capable of breaking down lactose into a fermentable sugar. If prompt fermentation takes place the sugar is presumably glucose, or possibly lævulose; if not, lactose or pentose, but the latter can be excluded by its color reactions. The dextrorotation of the lactose should also be determined by the polariscope.

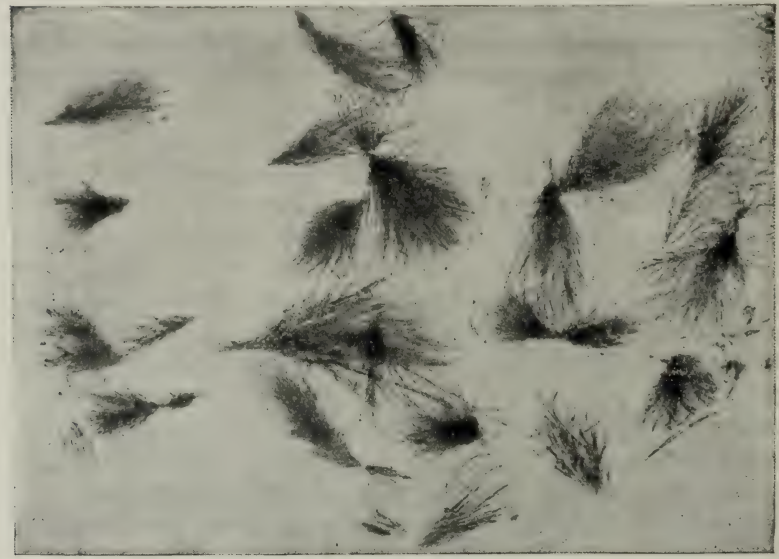

FIG. 148.—CRYSTALS OF PHENYLLACTOSAZON.

The phenylhydrazin test usually fails in urine, but if the specimen is concentrated and the residue extracted by heating with a considerable quantity of alcohol, filtering, and allowing the fluid to evaporate at room temperature, a relatively pure sugar will be obtained which gives a lactosazon (melting point, 200° C.), which shows certain morphological peculiarities which permits its differentiation from the other osazons. The crystals form small sheaves of very delicate curved needles looking like small tufts of silk threads, in contradistinction to the coarser straight needles of dextrosazon (Fig. 148).

Lactose also gives **Rubner's test.**[1] Three grams of lead acetate are added to 10 c.c. of the urine to be tested. The precipitate is filtered off and the filtrate heated in a test tube for three or four minutes until a brownish color appears. Ammonia is added and the heating continued, when if lactose is present a brick red color appears in the solution and a cherry red precipitate is deposited at the bottom of the tube, while the supernatant fluid becomes decolorized. Urines over 1.020 should be diluted with an equal bulk of water. The reaction is not very delicate, and usually is obtained only where the lactose content is from 0.3 to 0.5 per cent., hence it may be necessary to concentrate the urine and test the residue.

### MALTOSURIA

In a number of cases of disease of the pancreas, chiefly those with interstitial lesions, the presence of maltose in the urine has been determined. In a case reported by Rosenheim, with interstitial pancreatitis, the amount of sugar present varied from 0.1 to 0.5 per cent. In one of two cases reported by Kottmann,[2] in which glucose was found in the urine accompanied by small quantities of maltose, there was a calculus in the pancreatic duct. An autopsy was not obtained in the other, but the patient suffered from tuberculosis accompanied by diabetes. The identification of the sugar, however, was not very thoroughly carried out. Undoubted cases are those of le Nobel,[3] von Ackeren,[4] Flatow[5] and Lépine. In the case of von Ackeren there was a carcinoma of the head of the pancreas. It has also been shown that complete removal of the pancreas in dogs is accompanied by the excretion of maltose as well as dextrose. The determination of the presence of maltose depends upon the determination of the nitrogen content of the maltosazon, which contains 10.6 per cent. of nitrogen, and by the dextrorotation of 1° 30′ of a solution of 2 decigrams of the osazon in a mixture of 4 c.c. of pyridin and 6 c.c. of absolute alcohol. The melting point (207° C.) of the osazon is not sufficient to differentiate maltose from dextrose, as small amounts of impurity will result in a lowering of the melting point to that of the dextrosazon. The determination of the presence of the maltose in the urine is therefore chiefly of interest in the study of the diseases of the pancreas and has no great practical value.

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[1] Zeit. f. Biol., Bd. xx, 1884, p. 397.

[2] La Maltosurie: Thèse Inaug., Genève, 1901.

[3] Deut. Arch. f. klin. Med., 1888, Bd. xliii, p. 285.

[4] Berl. klin. Woch., 1889, p. 293.

[5] Berl. klin. Woch., 1898, p. 317.

## PENTOSURIA

The pentose group of sugars comprises those whose molecules contain five carbon atoms, in other words, $C_5H_{10}O_5$. The series is composed of eleven members, of which l-arabinose and l-xylose are of chief interest. These sugars, or an anhydride form, are abundantly present in all plant structures. The anhydride or pentosane is easily changed into the pentose form by heating with dilute acids, just as starch may be transformed into glucose by the same procedure. Pentose is also found in the nucleo-proteid of the tissues of the body, and in especial abundance in the pancreatic gland. By heating this tissue with dilute sulphuric acid l-xylose may be obtained. Only very small amounts of this sugar are present, however, in the body in this combination, probably not more than 10 grams.

Pure pentose when given by mouth or injected into the blood is rapidly excreted in unaltered form in the urine; but considerable quantities of pancreas or vegetable food may be ingested without the production of an alimentary pentosuria, probably because the slowness with which the sugar is set free permits its complete absorption and combustion. Traces of pentose, though usually not sufficient to reduce Fehling's, can be found in the urine after eating large quantities of vegetables or fruits containing an abundance of pentose, or by drinking liquors containing pentoses. The type of pentose present under these conditions is the dextrorotatory l-arabinose, a matter of considerable interest, as the form found in the urine in true pentosuria is an inactive r-arabinose.[1]

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[1] *Neuberg:* Chem. Berichte, Bd. xxxiii, 1900, p. 2243.

The racemic arabinose is probably excreted in combination with urea as an inactive arabinoseureid.

Traces of pentose have been demonstrated in the urine of a number of cases of severe diabetes; but this phenomenon is unimportant clinically, and may merely indicate that the lack of power of the tissues to oxidize glucose also extends to other forms of sugars. The exact form of the pentose excreted under these circumstances has not been determined.

Some thirteen cases of true pentosuria, not dependent upon the intake of large quantities of that sugar, have been described during the last decade. The patients gave no symptoms referable to diabetes. Some of them suffered from the morphine habit or had neurasthenia. One of the cases had a family history of diabetes, and another occasionally excreted small quantities of glucose though without diabetic symptoms. Brat[1] reports an interesting pair of cases in which brother and sister suffered from pentosuria. Bial[2] also reports three members of the same family all suffering from this disorder of metabolism. No deaths have occurred in persons known to be suffering from pentosuria, so that we have no knowledge of the pathological anatomy of the condition.

The amount of sugar excreted is small, rarely more than 10 or 20 grams per day, while in the best observed cases the excretion has been noted for at least six years without influence on the general health. The oxidizing power of the tissues for glucose has been shown not to be diminished, for 100 grams can be ingested without the production of an alimentary glycosuria. As the type of pentose found in the urine is the optically inactive r-arabinose, it can not be derived from the food which contains l-arabinose, nor from the nucleo-proteid of the body which contains l-xylose, and it seems probable that the sugar is formed synthetically in the body, possibly from galactose; but nothing more definite is known about the matter.[3]

**Tests for Pentose in the Urine.**—Urine containing pentose reduces Fehling's solution, but the copper oxide appears only after the mixture has been boiled for a few seconds, when there is a sudden general clouding of the fluid. The reaction thus differs from that produced by glucose and other sugars, where streaks of the oxide rise from the heated portion of the tube before the fluid begins to boil. This slow reduction is probably due to the fact that the pentose, as stated above, is presumably in combination with urea when excreted in the urine, and only reduces copper when set free by the action of heat and the alkali. A similar phenomenon is seen in the reduction of the glycuronic acid compound, urochloralic acid, the separation of the copper taking place only after boiling has continued for some little time.

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[1] Zeit. f. klin. Med., Bd. xlvii, 1903, p. 499.

[2] Berl. klin. Woch., 1904, p. 552.

[3] See *Bendix:* Die Pentosurie, Stuttgart, 1903. See also *Neuberg:* Ergebnisse der Physiologie, Bd. iii, Heft 1, 1904, p. 373.

Nylander's test usually gives a grayish or brownish deposit, and not as strong a black as is furnished by the other sugars.

Gas is not formed when pentose urine is fermented by yeast or pure cultures of saccharomyces. Pentose, however, can be broken up by impure yeast containing bacteria, but without the production of gas.

**Phloroglucin Test.**—Three c.c. of the urine are mixed with 3 c.c. of strong hydrochloric acid and a few granules of phloroglucin. The mixture is heated to the boiling point, and if pentose is present a cherry red color will appear throughout the fluid, which gradually changes to a dark green. If amyl alcohol is now added and the whole shaken, the coloring matter will be extracted and shows an absorption band between D and E. Lactose may give the color but not the absorption spectrum.

Diabetic urines and urines containing glycuronic acid also give this reaction, so that it is of slight value in testing complicated fluids like the urine, and is being replaced by the orcin test.

**Orcin Test.**—Three c.c. of the urine, preferably decolorized by filtration through animal charcoal, are mixed with 5 c.c. of hydrochloric acid of a specific gravity of 1.19 and a few grains of orcin are added. The fluid is brought nearly to the boiling point, but not over 95° C. If pentose is present a dark green color is produced and also a greenish blue precipitate which is characteristic of pentose. Diabetic urines and those containing glycuronic acid do not give the greenish blue precipitate. If amyl alcohol is added and the color shaken out an absorption band will be seen between C and D. A second band is often seen to the red side of C. An occasional band in the green is due to an excess of orcin.

Urines containing glucose do not give the band between C and D, and the color of the solution is usually yellowish or brown. Urines containing glycuronic acid often show the bands when the heating has been continued for too long a time. The reaction often fails even in the presence of pentose when the hydrochloric acid is not of sufficient strength. The presence of large amounts of glucose may interfere with the reaction, but if the glucose is removed by short fermentation with a pure culture of saccharomyces apiculatus, the pentoses will not be fermented and a strong reaction may be obtained. Fermentation with ordinary yeast is likely to remove the pentoses also.

**Bial's Test.**[1]—Ten c.c. of a solution composed of 500 c.c. of concentrated hydrochloric acid, 1 gram of orcin and 30 drops of 10 per cent. ferric chloride solution are brought to the boiling point in a test tube, and 5 c.c. of the urine to be tested are added. A green precipitate forms on cooling which can be further tested by dissolving in amyl alcohol and examining with a spectroscope. Pentose is present if a band is seen between C and D. If the urine and reagent are boiled for two or three minutes the conjugate glycuronic acid compounds will be split up and then give the orcin reaction.

**Phenylhydrazin Test.**—A pentosazon may be produced by heating the urine with phenylhydrazin hydrochloride and sodium acetate, and then allowing the mixture to stand in the cold for a considerable time. The crystals should be filtered off, recrystallized by dissolving in a small quantity of hot water and allowing to cool, and the melting point determined. The osazon of arabinose melts at from 166° to 168° C.[2] If dextrose is present in the urine the dextrosazon may be separated from the pentosazon by extracting with water at a temperature of 60° C. The glu-

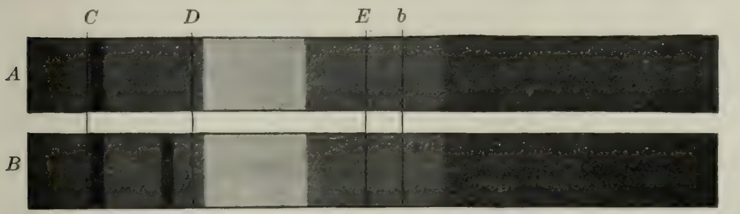

FIG. 149.—ORCIN TEST. *A*, Spectrum of Glucosazon ; *B*, Spectrum of Pentosazon or Pentose. (Blumenthal.)

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[1] Deut. med. Woch., 1902, p. 253.

[2] Blumenthal gives 155° to 160° C

cosazon under these conditions is practically insoluble, while the pentosazon dissolves freely in water of this temperature. The filtrate from the glucosazon crystals will then deposit the pentosazon when cooled, and if necessary concentrated somewhat by evaporation.

The osazons when submitted to the orcin test give characteristic spectra. (See Fig. 149.)

**Quantitative Determination.**—Inasmuch as the r-arabinose is inactive to polarized light it is impossible to estimate the sugar by the polariscope, and titration with Fehling's solution does not give accurate results. It is possible, however, to determine the arabinose by a method devised by Neuberg and Wohlgemuth.[1]

The approximate content of the urine in arabinose is determined by titration with Fehling's solution. If less than 1 per cent. is present, the urine must be concentrated in a vacuum until the sugar approximates 1 per cent.

One hundred c.c. of urine are mixed with 2 drops of 30 per cent. acetic acid, and evaporated on a water bath to about 40 c.c. Forty c.c. of hot 96 per cent. alcohol are then added and the mixture cooled. After standing for two hours the alcoholic solution is filtered from the urates and the inorganic salts which have separated, and the residue is carefully washed with 40 c.c. of 50 per cent. alcohol. One and one-fourth grams of pure diphenylhydrazin are then added to the filtrate and the mixture is warmed for a half hour on a water bath, the alcohol which evaporates being replaced. After the mixture has stood for twenty-four hours in a cold place the crystals which have separated are collected in a Gooch filter, using the filtrate to collect the crystals. The contents of the filter are then washed with 30 c.c. of 30 per cent. alcohol, and the whole is dried at 80° C. to a constant weight. The amount of arabinose is obtained by multiplying the weight of the hydrosazon by 0.4747.

### GLYCURONIC ACID

The interest in glycuronic acid from a clinical point of view lies chiefly in the fact that some of its conjugate compounds when present in considerable quantities in the urine are capable of causing a marked reduction of Fehling's solution with the separation of red copper oxide, and thus simulate one of the important sugar reactions.

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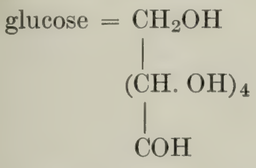
[1] Zeit. f. phys. Chemie, Bd. xxxv, 1902, p. 41.

Our knowledge of the conditions under which glycuronic acid appears in the urine and in the body fluids has been greatly increased in the last five years, chiefly owing to the investigations of Mayer and Neuberg. The isolation and identification of the acid previous to this time depended upon the separation from large quantities of urine, of pure compounds of the acid and required a complicated chemical technique. But the discovery by Neuberg[1] that the para-brom-phenylhydrazin compound of glycuronic acid is characterized by a high melting point (236° C.), insolubility in absolute alcohol, and an extraordinarily high lævorotation, has rendered the identification of glycuronic acid a comparatively simple process.

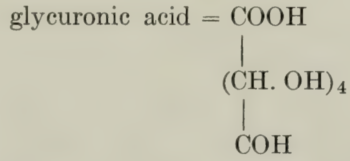
It has been shown by these observers that traces of glycuronic acid are present in normal urine in the form of phenol and indoxyl-glycuronic acids. It has also been shown that conjugate glycuronic acid is a constituent of normal blood,[2] and that a part of the substance called jekorin is undoubtedly a conjugate substance containing glycuronic acid. Lépine[3] has also shown that it is probable that in some cases in which an increase in the blood sugar has been assumed, the increase has actually been due to a high content of glycuronic acid.

The mode of origin of glycuronic acid in the animal organism has been generally assumed to be based upon a partial oxidation of grape sugar.

The chemical relationship of this compound with glucose can be seen by an inspection of their structural formulæ:



$$\text{glucose} = \begin{array}{l} CH_2OH \\ \quad | \\ (CH.\,OH)_4 \\ \quad | \\ COH \end{array}$$



$$\text{glycuronic acid} = \begin{array}{l} COOH \\ \quad | \\ (CH.\,OH)_4 \\ \quad | \\ COH \end{array}$$

It is evident that it is only necessary to substitute an oxygen molecule for two hydrogen molecules in the first group of glucose to produce glycuronic acid.

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[1] Chem. Berichte, Bd. xxxii, 1900, p. 2395.

[2] *Mayer:* Zeit. f. phys. Chem., Bd. xxxii, 1901, p. 518.

[3] Compt. Rend., tome cxxxiii, 1901, p. 138; and tome cxxxiv, 1902, p. 398.

The presence of conjugate glycuronic acid compounds in abundance in the urine may be regarded as an effort of the body to overcome an intoxication by combining with the substance which causes this intoxication.

The experiments of Hildebrandt,[1] which have shown that certain poisons can be neutralized in the animal body by a conjugation with glycuronic acid when large amounts of glucose are administered, point toward the truth of the oxidation theory. It is possible, therefore, that the presence of large amounts of glycuronic acid may prove of value in the clinical diagnosis of an intoxication.[2] An increase in this substance has been noted in hysteria, neurasthenia, and various neuroses, when all evidences of gastro-intestinal disturbance have been absent, though it is well known that in these conditions the indoxyl excretion may be higher than normal. An increased amount is also found in the urine of persons suffering from intestinal intoxication and from chronic heart disease, where the oxidizing power of the body is markedly reduced. In all these conditions it is probable that the increase in the acid is due to an increased formation of indol and other aromatic bodies in the intestine, and that an attempt is made by the organism to neutralize the toxic effects by conjugation with glycuronic acid.

Whether glycuronic acid can arise from other sources than the sugars, for example, from the albumin molecule, has not been proved. It is, however, very probable. But that portion of albumin which can be used to form glycuronic acid is in all probability the same which gives rise to glucose or glycogen, and therefore the question returns to the original point as to whether glycuronic acid is an oxidation product of glucose.

The most important drugs which incite an excretion of conjugate glycuronic compounds are chloral, chloralamid, butyl chloral hydrate, camphor, menthol, members of the turpentine group, sandalwood oil, phenol, acetanilid, antipyrin, phenacetin, naphthalin compounds, thallin, saccharin, salicylic acid, and alkaloids of the morphine and cocaine group. The chloral compound, urochloralic acid, is the one which is most frequently mistaken for sugar. The butyl chloral, camphor, phenol, and indoxyl compounds do not reduce Fehling's solution unless split by prolonged boiling of the strongly alkaline mixture. No one of these compounds ferments with yeast. It should be remembered that chloralamid if given in large doses may incite a glycosuria.

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[1] Arch. f. exp. Path., Bd. xliv, 1900, p. 278.

[2] For an interesting case see *Wohlgemuth:* Berl. klin. Woch., 1904, p. 1084.

Diabetic urine frequently contains considerable quantities of conjugate glycuronates, and after the glucose has been removed by fermentation a slight lævorotation may appear. This lævorotation, however, may be due to β-oxybutyric acid, but as this acid is not precipitated from urine by lead subacetate or lead subacetate plus ammonia, the lævorotation due to the glycuronic acid can be removed by precipitating the urine with the above-mentioned salts of lead, and the β-oxybutyric lævorotation will still remain in the filtrate. The precipitate formed on the addition of the lead subacetate can be decomposed by hydrogen sulphide, and the glycuronic acid compounds set free can be recognized by giving a strong orcin reaction when heated with hydrochloric acid and a few granules of orcin. Or the β-oxybutyric acid may be removed from the urine by shaking out that fluid three or four times with considerable quantities of ether, and the remaining fluid may be tested by the polariscope or orcin.

Care must be taken in determining the lævorotation due to glycuronates to keep the urine acid, as with an alkaline reaction these substances are inactive. Small amounts of lævorotation may be disregarded, as urine normally contains sufficient lævorotatory substances to produce a rotation of one-tenth of a degree. If any amount of the glycuronic compounds are present, the rotation will be over two-tenths of a degree.

### TESTS FOR GLYCURONIC ACID

Inasmuch as the glycuronates give the same reactions as the pentoses, if the fluid containing them is boiled for a short time with hydrochloric acid, and especially if Bial's modification of the orcin test is used, this color reaction becomes of comparatively slight value in the final identification of the glycuronates. If, however, pentoses are absent, Bial's reagent is useful.

**Bial's Test.**[1]—A stock solution composed of 500 c.c. of strong hydrochloric acid, 1 gram of orcin, and 25 drops of 10 per cent. ferric chloride, is kept for the test.

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[1] Die Diagnose der Pentosurie, Deut. med. Woch., 1902, p. 253; also Zeit. f. klin. Med., Bd. xlvii, 1902, p. 489, and Bd. 1, 1903, p. 417.

Five c.c. of the reagent and 3 c.c. of the urine to be tested are placed in a tube and warmed over a flame until bubbles begin to rise, but not boiled. If a green precipitate forms on cooling, which on further testing by solution in amyl alcohol and examination with a spectroscope shows characteristic absorption bands, the presence of pentoses may be assumed. If, however, the urine and reagent must be boiled for several minutes in order to get the reaction, the conjugate glycuronic acid compounds which are thus split up will give the green precipitate and the characteristic absorption spectrum.

This spectrum is characterized by an absorption band between the lines *C* and *D*. Only a strong reaction is diagnostic, and there is reason to believe that it may occasionally be given by other sugars.[1]

**Phenylhydrazin Test.**—Glycuronic acid or the conjugate glycuronates do not form an osazon by the ordinary methods of heating for a short time on a water bath. An osazon may, however, be formed by heating with phenylhydrazin and acetic acid for several days in an incubator at 40° C. The osazon so formed crystallizes in bundles of yellowish needles of a melting point of 200° to 205° C. The compound is only slightly soluble in water, but dissolves easily in pyridin and shows lævorotation in this solution. Its melting point and morphology are so similar to those of the glucosazon that the formation of this compound is of no interest from a diagnostic standpoint.

**Neuberg's Test.**—The identification of glycuronic acid can only be made with certainty by the method of Neuberg.[2] The urine to be examined is heated in an autoclave for one to two hours at a temperature of 115° C., with sufficient sulphuric or phosphoric acid so that the fluid contains from 1 to 2 per cent. After cooling, the mixture is neutralized with sodium carbonate and immediately acidulated with acetic acid and filtered. Two hundred and fifty c.c. of the filtrate are mixed with a hot aqueous solution of 5 grams of para-brom-phenylhydrazin hydrochloride and 6 grams of sodium acetate. The mixture becomes cloudy after the addition of the reagent, but on further heating the cloud disappears and in five to ten minutes needle-shaped crystals are formed. The fluid should be allowed to cool and the crystals filtered off. The filtrate is again heated, and after cooling the second crop of crystals is removed. This should be repeated four or five times. The collected crystals are washed with water and *absolute* alcohol, and then recrystallized several times from 60 per cent. alcohol in which they are soluble. The melting point of the para-brom-phenylhydrazin compound is 236° C. Besides the melting point, the lævorotation of the osazon when in solution may be used to determine the presence of glycuronic acid. Two decigrams of the substance dissolved in a mixture of four parts of pyridin and six of absolute alcohol give a lævorotation of 7° in a 10 cm. tube.

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[1] See for a criticism of the method, *Leersum:* Beit. z. chem. Phys. u. Path., Bd. v, 1904, p. 510.

[2] Chem. Berichte, Bd. xxxii, 1900, p. 2395.

## ACETONE BODIES

In the urine of diabetics three substances are found in varying proportions and so closely connected chemically that it is important to consider them together. These are:

$CH_3 - CHOH - CH_2 - COOH$ or $\beta$-oxybutyric acid.
$CH_3 - CO - CH_2 - COOH$ or diacetic acid.
$CH_3 - CO - CH_3$ or acetone.

It is now rather generally assumed that the oxybutyric acid is the fundamental substance from which the others are produced by progressive oxidation toward the final results of the metabolism of these bodies, $CO_2$ and $H_2O$. The fats are in all probability the source of these substances, though the older idea that they are derived from the proteids of the body (not those of the foods) has not been entirely given up.

The reason for this latter assumption is that when the carbohydrates are withdrawn from the food of either a diabetic or a normal person, and the body then becomes dependent upon the proteids for its sugar, the excretion of acetone immediately increases from the small amount normally found in the urine to a very considerable quantity. This explanation of the derivation of the acetone from the proteids has been found to be possible for normal persons, but diabetics may excrete more acetone than can be accounted for by the amount of proteid broken down, as determined from the nitrogenous excretion in the urine. The ingestion of fats may also cause an increase in the acetone excretion. The fats are presumably the source of the excess, and the

TABLE OF CARBOHYDRATE REACTIONS

| | Fehling's solution | Ten c.c. of Fehling's is equal to | Nylander's solution | Phenylhydrazin | Melting point of osazon | Two dgms. of osazon dissolved in 6 c.c. of absolute alcohol and 4 c.c. of pyridin gives rotation in 10 cm. tube | Polarization | Fermentation with yeast | Other tests |
|---|---|---|---|---|---|---|---|---|---|
| Glucose | Positive | .05 grams | Positive | Positive | 205° | $-1° 30'$ | $[\alpha]^{20}_{D} = +52.6°$ | Positive | |
| Lævulose | Positive | .05 grams | Positive | Positive | 205° | Osazon $+1° 20'$<br>Methylphenylosazon $+1° 40'$ | $[\alpha]^{20}_{D} = -92°$ | Positive | Seliwanoff reaction |
| Maltose | Positive | .0719 grams | Positive | Positive | 207° | $+1° 30'$ | $[\alpha]^{20}_{D} = +138°$ | Positive | Does not react to Barfoed's reagent |
| Lactose | Positive | .067 grams | Positive | Negative | 200° | Inactive | $[\alpha]^{20}_{D} = +52.5°$ | Negative | |
| Pentose | Positive | .0542 grams | Only a faint reaction | Positive | 168° | Inactive | Inactive | Negative | Positive to orcin test |
| Glycuronic acid | Occasionally positive | Varies with different compounds | Occasionally positive | Negative | 200–205° (p-bromphenylosazon 236°) | p-bromphenylhydrazin compound $-7°$ | Left (varies with different compounds) | Negative | Positive to orcin test after decomposition of the conjugate compound with strong acid |

amount of production of acetone bodies seems to depend upon the lack of ability of the body to use the carbohydrates.

So long as the body is able to oxidize a sufficient amount of carbohydrate the acetone remains low, and if the urine of a diabetic contains much acetone the amount can be reduced by giving carbohydrates ; but when the body is no longer capable of oxidizing the carbohydrates for its own use, then the amount of oxybutyric acid and acetone begins to increase.

The amount of diacetic and $\beta$-oxybutyric acids formed can be indirectly estimated by a determination of the ammonia in the urine, since the oxybutyric and the diacetic acid circulating in the blood and tissues combine with ammonia and the resulting compounds are excreted through the urine. The normal excretion of ammonia is about 0.7 grams. If 2 grams are found in the urine it is equivalent to about 6 grams of oxybutyric acid, 5 grams to 20 and 8 grams to 40 grams of oxybutyric acid. In diabetics the determination of the presence or absence of the three acetone bodies is of far greater importance, from a point of view of prognosis, than the determination of the amount of sugar excreted.

The milder cases of diabetes, while still on a mixed diet, may show no increase in the amount of acetone excreted and no oxybutyric and diacetic acid. If, then, these patients are put on a diet free from carbohydrate, the amount of acetone will increase and a trace of diacetic acid make its appearance, but no oxybutyric. This condition may last for a few weeks, and then the diacetic acid will disappear from the urine and the acetone return to nearly normal amounts ; and this change is quite permanent, especially if the patient gradually returns to a diet containing a small quantity of carbohydrate. The prognosis in all such cases is exceedingly good.

In the severe forms, on the other hand, the urine constantly contains all three of the acetone bodies and increased ammonia, and these persist in the urine without being much influenced either by reduction of the carbohydrates or by their use. These patients may be excreting large quantities of sugar, or the amount may be exceedingly small. Such cases are, as a rule, of very bad prognosis, though the exact time at which death will occur can not be judged from the amount of the acetone bodies in the urine. Some patients survive for months, or even years, with very large quantities of these substances in the urine; others die within a few weeks after the increased formation of these bodies begins.

The intermediate cases are those which show a moderate excretion of acetone and the acids, but which may be so influenced by a reduction of the carbohydrates as to cause these substances to disappear. It may be necessary to give these patients a moderate amount of carbohydrate to free the urine entirely from the acid excretion, but in general it may be assumed that, with treatment, the urine may be rendered normal so far as the excretion of the acetone group is concerned. Glucose may persist in larger or smaller quantities. The prognosis in these cases depends solely upon the ease with which the excretion of the acetone group can be controlled; if this is possible the prognosis becomes relatively good.

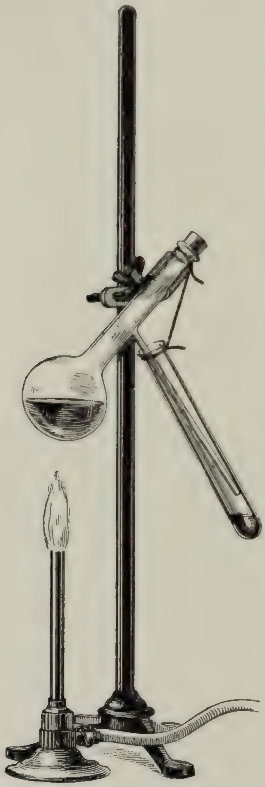

Fig. 150.—Simple Distilling Apparatus.

ACETONE

Acetone may appear in the urine in other conditions besides diabetes. Thus it has been observed in those who are suffering from starvation; i. e., patients with carcinomata of the stomach or the œsophagus which cause complete obstruction. It has been frequently found after ether or chloroform narcosis[1] and in many digestive disorders, especially in children. It is also present for a few days before and after delivery. In patients with fever a small amount of acetone is not uncommon, and it is liable to be especially abundant in the febrile conditions of children and more often in the exanthemata.

**Tests for Acetone.**—The urine for these tests should be distilled, as the other substances which are present interfere with the reactions. The obtaining of a few cubic centimeters of distillate requires only a short time and the simplest apparatus. The best way is to use a flask with a tube extending obliquely out from the neck, a so-called distilling flask; and if, after the urine has been added, the neck is closed with a stopper and a test tube is fastened over the end of the side tube with a piece of string or a clamp, enough distillate will collect in two minutes for all the tests. No cooling apparatus is necessary.

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[1] For a study of a series of fatal cases of acetonuria following anæsthesia, see *Brackett*, *Stone*, and *Low:* Bost. Med. and Surg. Jour., vol. cii, 1904, p. 2.

**Legal's Test.**—A few drops of a freshly prepared solution of sodium nitroprusside are added to a small amount of the distillate, or even to the urine, and the whole is then rendered alkaline with sodium hydrate. A red color which changes on the addition of acetic acid to a carmine and then to a purple-red and finally into violet shows the presence of acetone. This is not so decisive a test as the following:

**Lieben's Test.**—A little Gram's solution is added to the distillate and then some sodium hydrate. If acetone is present in large quantity there will be an immediate separation of a yellow precipitate of iodoform. If the quantity is smaller the iodoform is not visible to the naked eye, but the deposit which collects in a short time at the bottom of the test tube should be examined microscopically for the crystals of iodoform. As a rule, small quantities may also be recognized by the odor if the test tube be warmed. Alcohol gives the same reaction.

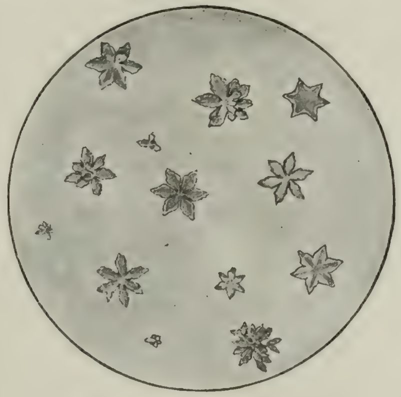

FIG. 151.—IODOFORM CRYSTALS. Magnified 500 diameters.

**Gunning's Test** does not react to alcohol, but it is less convenient, as it requires twenty-four hours for the precipitate of iodide of nitrogen to disappear. An alcoholic solution of iodine and some ammonia are added to the distillate and the mixture set aside until the heavy black precipitate of the iodide of nitrogen has disappeared and the iodoform deposit can be recognized and examined under the microscope. Do not warm the mixture when any iodide of nitrogen is present in the test tube.

### DIACETIC ACID

The presence of diacetic acid in the urine is usually an evidence of a grave disorder of metabolism. It appears in the latter stages of the same diseases which show acetone in the urine—that is, in malignant diseases of the stomach and intestines, in the exanthemata, and occasionally in the acute degenerations of the liver, etc. It usually precedes the appearance of oxybutyric acid as acetone has preceded it. Diacetic acid is very volatile and disappears from the urine in from twenty-four to forty-eight hours, so that the tests should only be carried out on perfectly fresh urine.

**Tests for Diacetic Acid.**—1. A considerable quantity of a 10 per cent. solution of ferric chloride is added to a fresh specimen of the urine. The first few drops of the iron salt produce a considerable yellowish precipitate of ferric phosphate, so that the addition of the reagent must be continued until all the phosphates are removed; then if diacetic acid is present a further addition of the ferric chloride will produce a deep reddish brown color, the so-called "Bordeaux red reaction," or as it is also called, Gerhardt's reaction.[1] If the amount of diacetic acid is small the phosphate precipitate should be filtered off and the filtrate tested by further addition of ferric chloride. The color due to diacetic acid remains only for a few hours, as the acid quickly decomposes into acetone and carbonic acid even at room temperatures, hence the necessity of using perfectly fresh urine in testing for this substance. This change takes place much more quickly at high temperatures, a phenomenon made use of as a means of distinguishing between the color due to diacetic acid and that caused by certain drugs excreted in the urine, most of the drug reactions not being affected by heating. The substances which give a color similar to that caused by diacetic acid are the sulphocyanides, salts of formic and acetic acid, phenol, salicylates, and the products excreted in the urine after the ingestion of phenacetin, antipyrin, thallin, kryofin, and kairin. When there is any doubt concerning the nature of the reaction, 10 c.c. of urine are to be acidulated with sulphuric acid and shaken up in a test tube with an equal bulk of ether. The ether is removed by means of a pipette to a clean test tube and again shaken up with a few c.c. of very dilute ferric chloride. If diacetic acid is present the water assumes a violet color. If the urine contains salicylic acid a similar color is produced. It is possible to remove the salicylic acid by shaking out with benzol or chloroform before extracting with the ether, but it is much simpler to use the following test.

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[1] *Gerhardt:* Wien. med. Presse, 1865, p. 674.

2. Arnold[1] has devised a test for diacetic acid which is more delicate than the Gerhardt, and is not interfered with by any of the drug reactions. Two solutions are to be kept in stock. One is a 1 per cent. aqueous solution of para-amido-acetophenon with 2 c.c. of strong hydrochloric acid in each hundred of the mixture; the other is a 1 per cent. solution of potassium nitrite. Two parts of the first and one of the second solution are mixed together in a test tube and an equal bulk of urine added, and finally a drop of strong ammonium hydrate. A brown color appears even in normal urines, but if an excess of strong hydrochloric acid be added, the normal urine changes to a yellow color, while that containing diacetic acid assumes a beautiful purple color and on shaking shows a violet foam, a point on which Waldvogel[2] lays great stress, as normal urines may occasionally show a red color. If the urine contains much pigment it should be decolorized by filtration through animal charcoal. Lipliawsky[3] has modified the Arnold reaction and increased its delicacy. The same stock solutions are used—that is, 6 c.c. of the first, 3 c.c. of the second, and 9 c.c. of the urine to be tested. A drop of strong ammonia is added and the whole thoroughly shaken, during which process a brick red color appears. Ten drops to 2 c.c. of the mixture of urine and reagent are then removed to another tube and 20 c.c. of strong hydrochloric acid and 4 drops of ferric chloride solution are added, together with 3 c.c. of chloroform. The corked test tube is then slowly tilted from side to side so as not to emulsify the chloroform. At the end of a minute, if diacetic acid is present, the chloroform will assume a violet color. In normal urines the color will be either a yellow or a pale red. Salicylic acid and similar drugs do not interfere with the reaction.

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[1] Wien. klin. Woch., 1899, p. 541; also Cent. f. inn. Med., 1900, Bd. xxi, p. 417.

[2] Die Acetonkörper, Stuttgart, 1903.

[3] Deut. med. Woch., 1901, p. 151.

## BETA-OXYBUTYRIC ACID

β-oxybutyric acid is found in the urine most frequently in diabetes, and is almost always accompanied by diacetic acid and acetone, although the latter may be present only in small quantities if the urine is perfectly fresh. As much as 80 gms. of the acid may be excreted in twenty-four hours in a severe case of diabetes. The acid has also been found in the urine in cases of scarlet fever, measles, and scurvy, in many of the acute infectious diseases of children, in the so-called recurrent vomiting of children, in some types of eclampsia with persistent vomiting, in melancholic patients who refuse food, and also in perfectly healthy individuals, especially those who have an abundance of fat and who either change suddenly to a pure protein diet or else go without food for several days.

**Qualitative Tests for β-Oxybutyric Acid.**—It is usually stated that if the urine is fermented with yeast to remove the glucose, and then examined with a polariscope and found to show a lævorotation, the presence of β-oxybutyric acid is probable. While this is to a certain extent true, yet the lævorotation may be due to glycuronic acid, and small quantities of β-oxybutyric acid do not give sufficient rotation to make their presence known. The following procedures are, therefore, more satisfactory:

**Method of Hart.**[1]—To 20 c.c. of the suspected urine, add 20 c.c. of water and two drops of acetic acid, and boil until the volume is reduced to about 10 c.c. Make up with water to 20 c.c., and divide the mixture equally between two test tubes. To one tube add 1 c.c. of hydrogen peroxide, and warm gently for about one minute, and then allow to cool. Add to each test tube 0.5 c.c. of glacial acetic acid and a few drops of a freshly prepared watery solution of sodium nitroprussiate. Overlay the solution in each test tube with 2 c.c. of concentrated ammonium hydroxide. Allow the tubes to stand for four hours. At the end of this time compare. At the point of contact between the reagent and the fluid, the control tube will show no ring, or only a faint brown if much creatinin be present. The tube to which the peroxide was added will show a purplish red contact ring if β-oxybutyric acid was originally present. If none of this acid is in the urine the two tubes will show no difference. If both are now shaken, the difference in color will be seen throughout the fluid. The phenomenon is more marked if the tubes stand for fifteen minutes after shaking. The presence of sugar does not interfere with the reaction. Albumin should be removed. The method will detect 0.3 per cent. of β-oxybutyric acid.

**Method of Black.**[2]—From 10 to 50 c.c. of urine are concentrated in an evaporating dish over a water bath to one-fourth the original volume.

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[1] Amer. Jour. Med. Sciences, cxxxvii, 1909, p. 869.

[2] Jour. of Biological Chemistry, v, 1908, p. 207.

The residue is acidified with a few drops of concentrated hydrochloric acid, and made into a thick paste with plaster of Paris, and then allowed to stand until it begins to set. The plaster is then broken up in a dish with a blunt glass rod. The coarse meal thus obtained is extracted with ether for two hours. The ether extract, which contains the β-oxybutyric acid, is evaporated over a water bath; the residue is dissolved in water and neutralized with barium carbonate, and two or three drops of commercial hydrogen peroxide are added, and then a few drops of 5 per cent. ferric chloride containing a trace of ferrous chloride. On standing a rose color develops which gradually fades owing to further oxidation of the diacetic acid. The solution should be cold and approximately neutral; and an excess of the peroxide and iron should be avoided. The test shows 1 part of β-oxybutyric acid in 10,000.

**Crotonic Acid Method.**—For the final identification of β-oxybutyric acid by any of the tests given in this section, it is advisable to attempt to form and isolate α-crotonic acid. If large amounts of β-oxybutyric acid are present, the crotonic acid may be obtained directly from the urine by fermenting that fluid to remove glucose and then evaporating to a small bulk and distilling with an equal volume of 50 to 55 per cent. sulphuric acid (sp. gr. 1.40 to 1.45). The volume should be kept constant by the addition of water in order that the sulphuric acid may remain of the same concentration. The distillate should be chilled on ice and the α-crotonic acid will often crystallize out. If it does not, the distillate can be shaken out with ether and the residue left after evaporation tested by determining its melting point, which should be from 70° to 72° C. Benzoic acid, which may go over in addition, is distinguished by a melting point of about 121° C.

**Quantitative Determination of β-Oxybutyric Acid.**—It is impossible to determine accurately the amount of β-oxybutyric acid in the urine by the polariscope, it is, therefore, necessary to separate the acid from the urine.

**Method of Magnus Levy.**[1]—This method gives the best results when about 1 gram of β-oxybutyric acid is obtained in the final extraction. It is, therefore, necessary to guess at the amount of oxybutyric acid present by the depth of the Black reaction or by a polariscope test of the urine. To each 100 c.c. of the fresh urine are added 40 grams of ammonium sulphate and 15 c.c. or 20 per cent. sulphuric acid. The urine is then treated in an extraction apparatus with ether. The most efficient type is that of Zelmanowitz,[2] though the Kossel form is excellent. The extraction requires from twenty-four to seventy-two hours, depending upon the rapidity with which the ether is passed through the fluid. If in doubt, extract for twenty-four hours in addition and by one of the qualitative tests on a small portion of the fluid, determine whether any β-oxybutyric acid is present. The ethereal extract is poured through a dry filter into a dish and allowed to evaporate at room temperature. Hippuric acid crystals usually separate. About 15 c.c. of water is then added and the whole is allowed to stand in order to permit separation of an oily precipitate. The residue is washed with a very small amount of water and the fluid and washings are made up to 10 or 20 c.c. A small quantity of silicious earth is added and the fluid is filtered through paper until perfectly transparent. The rotation is then determined. The specific rotation of $\beta$-oxybutyric acid is $[\alpha]$—24.12.

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[1] Ergebn. der Med., I, 1908, p. 417. [2] Biochemisches Zeit., I, 1906, p. 253.

The amount of $\beta$-oxybutyric acid in grams in 100 c.c. of the solution examined is obtained by this formula: $g = \frac{100 \cdot a}{l\,[\alpha]}$ where $a$ is the rotation in degrees and decimals of the fluid tested, $l$ the length in decimeters of the tube employed, and $[\alpha]$ the specific rotation of $\beta$-oxybutyric acid, in this case—24.12.

**Method of Black.**—The qualitative test of Black, given above, can also be applied quantitatively. An aliquot part of the twenty-four hour urine, approximating 50 to 100 c.c., is taken, made faintly alkaline with sodium carbonate, and boiled to one-third of its volume, and further concentrated to about 10 or 20 c.c. on a water bath. The residue is cooled, acidified with a few drops of hydrochloric acid, and mixed with plaster of Paris. The granular meal obtained by breaking up the plaster is transferred to a Soxhlet apparatus and extracted for two hours with ether. The extract is evaporated spontaneously. The residue is dissolved in water, decolorized, if necessary, with bone black, filtered, and diluted to a known volume. The $\beta$-oxybutyric acid is then determined by the polariscope.

### DIAZO-REACTION OF EHRLICH[1]

Many substances are found in the urine of healthy persons which will combine with the so-called diazo bodies and give rise to highly colored pigments. Among these is oxyproteic acid. If, however, these diazo bodies are used with certain reagents and in proper dilution only, no such general reaction takes place and its appearance is confined to pathological urines, and also to the urines from a limited number of diseases. Two solutions are required:

| | | |
|---|---|---|
| I. | Sulphanilic acid | 1 |
| | Hydrochloric acid (concentrated) | 50 |
| | Water | 1000 |
| II. | Sodium nitrite | 1 |
| | Water | 200 |

The solutions keep best in dark glass bottles. To obtain the reagent 50 c.c. of I is mixed with 1 c.c. of II. The mixed fluids keep for about two days in warm weather, somewhat longer in cold. Equal quantities of the combined reagents and the urine to be tested are mixed in a test tube and one-eighth to one-tenth of the bulk of ammonia added and the whole thoroughly shaken. The ammonia should be added quickly, not drop by drop.

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[1] A very complete monograph on this subject is that by *Clemens:* Deut. Arch. f. klin. Med., Bd. lxiii, 1899, p. 74 (Bibliography).

Para-amido-acetophenon may be substituted for the sulphanilic acid and is preferred by some observers, as it gives a somewhat darker shade of red, thus bringing out faint reactions more clearly than the substance originally suggested by Ehrlich.

A positive reaction is obtained when the fluid takes on a deep cherry red color and the supernatant foam shows a bright salmon pink. In a well-marked reaction the foam may be of quite a deep red color.

On standing twenty-four hours a green precipitate will be found at the bottom of the test tube which is characteristic of the true diazo-reaction, but is not constant in all cases, and is not necessary for a positive reaction. Many drugs, such as naphthalin and chrysarobin, when taken internally, give a reaction which is quite similar in appearance to the true diazo-reaction, but can, as a rule, be distinguished by the fact that the color is more permanent in alkaline solutions, does not fade to any extent on the addition of a strong mineral acid, the foam is more yellow than in the typhoid reaction, and the green precipitate does not appear on standing. Still more important, especially in connection with cases of phthisis, is the fact that the internal administration of gallic or tannic acid, tannigen, tannalbin, phenol, or salol, or the opium alkaloids, diminishes the diazo-reaction, or even causes it to disappear. The same is true of the iodides. Inasmuch as the tannic acid compounds are frequently administered in phthisis to influence the diarrhœa so common in such cases, the use of the diazo-reaction to aid in prognosis should be carefully controlled by a knowledge of the patient's medication. Creosote and creosotal also cause a great diminution in the intensity of the reaction or may even suppress it. Clemens[1] has also shown that the presence of bile, urobilin, or hydrochinon in the urine, may interfere with the reaction. In such cases he recommends the removal of the pigment by the addition of a few drops of lead acetate solution or a little animal charcoal and then filtering. It is often possible to obtain a reaction by shaking out the acidified urine with ether or amyl alcohol, neither of which extracts the diazo-substance.

The intense yellow reaction which we often meet with in the urine of phthisical patients has been shown by Burghart[2] to be due to phenol which is often present in excess in the urine of the severe cases and as a rule completely obscures the reaction. The excess of phenol in such urines can be easily demonstrated by adding some strong nitric acid to a test tube of the urine and boiling. After the mixture has cooled bromine water is added, and if a marked turbidity of the urine is produced an excess of phenol compounds may be assumed. This excessive production and excretion of phenol compounds is often observed in severe cases of phthisis, and often accounts for the frequent absence of the reaction at that time. Cases, however, of excessive phenol excretion have been observed in which the diazo-reaction could still be easily obtained, so that other causes must play some part. One possibility is that the excretion of the diazo-substance in the urine takes place irregularly, as has been noted in the normal excretion curve of other substances, notably urea.

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[1] Deut. Arch. f. klin. Med., Bd. lxiii, 1899, p. 74.

[2] Berl. klin. Woch., 1901, p. 276; also Deut. med. Woch., Vereins-Beilage, 1901, p. 195.

The value of the diazo-reaction lies in the fact that it is present in the first two weeks of typhoid fever in the urine in from 70 to 80 per cent. of the cases, exceeding in this respect the Widal reaction. It usually reappears in a relapse when the agglutination test is useless. The intensity of the reaction is, as a rule, parallel with the intensity of the infection, while the Widal is often best marked in the lighter cases. The reaction usually disappears at the end of the third week of the disease. The clinical value of the diazo-reaction in the diagnosis of typhoid fever is somewhat weakened by the fact that it occurs in well-marked form in acute miliary tuberculosis, in pneumonia, and occasionally in many of the acute exanthemata. During a complicating pneumonia the reaction often disappears from the urine.

The diazo-reaction is found in from 10 to 15 per cent. of persons with acute lobar pneumonia and acute serous pleurisy. Measles gives a positive reaction in nearly all the cases, scarlatina in about one-third. The diazo is absent in rötheln.

The continued presence of the diazo-reaction in persons without fever points to tuberculosis. In cases of phthisis with a well-marked diazo-reaction the prognosis is, as a rule, distinctly bad, so much so that the reaction has been used as a convenient method to decide upon the advisability of climatic treatment in patients suffering with advanced phthisis. But as the writer has shown [1] some 10 per cent. of the cases showing a strong diazo on the first examination are capable of cure by the proper care. A considerable per cent. however of patients with a strong positive reaction die within six months, despite treatment of any sort.

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[1] Prognostic Value of the Diazo-Reaction in Pulmonary Tuberculosis, Medical News, vol. lxxxii, 1903, p. 631.

The reaction is often absent in active cases of tuberculosis when a lesion of the kidney exists. If the nephritis becomes less acute the diazo may again be found in the urine.

In tuberculous peritonitis, the reaction is often found, which differentiates this disease from cirrhosis of the liver in which the reaction is not present. As the reaction is occasionally noted in the urine of persons suffering from new growths involving the peritoneum, a differential diagnosis between this condition and tuberculous peritonitis is not possible from the diazo-reaction alone.

It will be seen from the foregoing statements that the diazo-reaction is one of the important symptoms of typhoid fever, but that its absence does not negative the clinical diagnosis of typhoid fever, nor does its presence indicate that the patient is necessarily suffering from that disease. In a suspected relapse the presence of the reaction is of diagnostic value.

## THE URINARY PIGMENTS

In normal urine two pigments are always present : urochrome and hæmatoporphyrin. Two others may be present, especially after standing : uroerythrin and urobilin. The first mentioned pigment gives the yellow color to the urine; the second is red, and present only in small amounts; the third gives the pink color to the urate deposits. Urobilin colors the urine a dark yellow brown. Under pathological conditions, hæmoglobin, methæmoglobin, and the bile pigments are the chief urinary coloring matters. Hæmatoporphyrin is considered under the head of hæmaturia.

**Urochrome** is the name given to the yellow pigment which gives the natural amber color to the urine. It can be isolated as a brown, easily soluble powder which furnishes yellow solutions in water and alcohol. No definite absorption bands are present when its solution or normal urine is examined spectroscopically. It is precipitated by phosphotungstic and phosphomolybdic acids and by heavy metals. Advantage is taken of this property in clearing the urine for examination by the polariscope; lead acetate is added to the urine, and the insoluble precipitate thus formed is filtered off, leaving a perfectly clear, colorless solution. This pigment is closely related to urobilin and is derived from the bile coloring matters.

**Urobilin,** or its antecedent, urobilinogen, is often present in small quantities in normal urines. If not present in the urine when freshly passed, it soon appears from the breaking down of the urobilinogen by the action of the air and light. The latter substance may also be transformed into urobilin by the action of acids. It is formed by the reducing action of the intestinal bacteria on the bile pigments and is generally considered as identical with the stercobilin of the fæces. It appears in quantity in the urine in infectious diseases, in atrophic cirrhosis of the liver, in lead colic, and in uncompensated heart disease. It is very abundant in the urine of pernicious anæmia or any disease accompanied by marked destruction of the blood or hæmorrhage into any of the organs. Urobilinuria has been seen after the administration of antifebrin, antipyrin, after chloroform inhalations, and the injection of tuberculin. All of these substances are capable of destroying the red blood corpuscles. In advanced nephritis, urobilin may be retained in the blood by the impermeability of the diseased renal epithelium for this substance. It may in extreme cases give a brownish yellow color to the urine and a yellow tint to the foam, so that bile pigment may be suspected. It often appears before the bilirubin of an obstructive jaundice can be obtained from the urine and may alternate with that substance; that is, the urine may contain large quantities of the bile pigment for a time and then only urobilin; later the bilirubin may appear. This urobilin icterus is most often seen in atrophic cirrhosis of the liver, and occasionally in pneumonia and carcinoma. The urobilin may be demonstrated in the blood under these conditions.

**Tests for Urobilin.**—1. Add to the urine 2 to 5 drops of a 10 per cent. solution of zinc chloride, then enough ammonia to dissolve the precipitate of zinc oxide. Filter from the precipitate of urinary phosphates and hold the test tube against a dark background. If any quantity of urobilin is present a green fluorescence will be seen in the fluid. Schlesinger recommends adding to the urine an equal bulk of a 1 per cent. alcoholic solution of zinc acetate and filtering the mixture. The fluorescence is very marked, especially if the light be concentrated by a lens.

2. A better method is to extract 50 c.c. of the urine with 50 c.c. of pure ether, evaporate the ether, and dissolve the brown residue in a little strong alcohol. The solution will be pale yellow with a green fluorescence.

3. Ten to 20 c.c. of the suspected urine are acidified with several drops of HCl and shaken gently with 5 c.c. of amyl alcohol. The amyl alcohol extracts the pigment and shows a bright green fluorescence when treated with an alcoholic solution of zinc chloride and a little ammonia. By transmitted light the amyl alcohol extract is a faint pink color.

4. Urobilin, when present in large quantities in the urine, gives the biuret test, and so can be confused with albumin or albumose. As it is also precipitated by phosphotungstic acid with the albumose, it may invalidate the Hofmeister-Salkowski test for albumose. To avoid confusion, either test the urine for urobilin and use small quantities (10 c.c.) for the albumose test, or, better, wash the urobilin out of the phosphotungstic precipitate with alcohol.

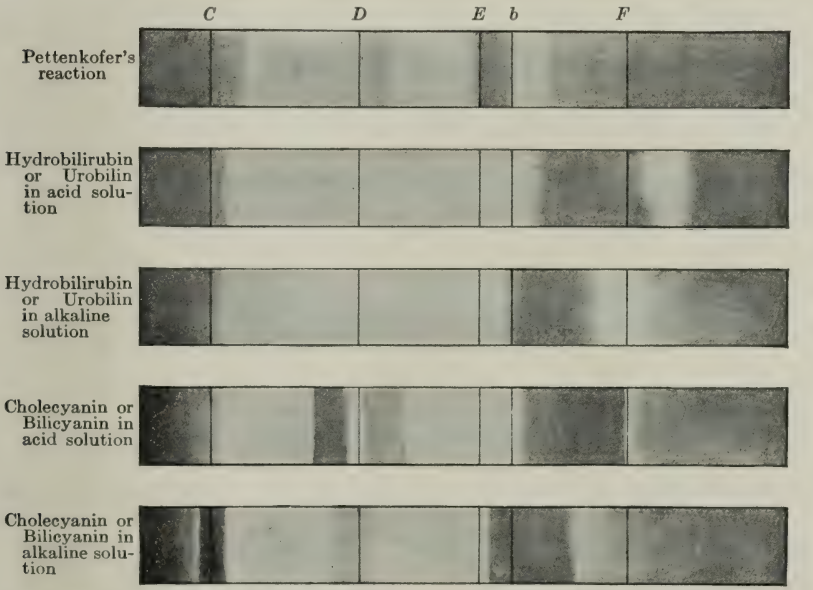
C
D
E
b
F
Pettenkofer's reaction
Hydrobilirubin or Urobilin in acid solution
Hydrobilirubin or Urobilin in alkaline solution
Cholecyanin or Bilicyanin in acid solution
Cholecyanin or Bilicyanin in alkaline solution

FIG. 152.—ABSORPTION SPECTRA OF PETTENKOFER'S REACTION AND OF BILE PIGMENTS.

5. The most rapid method of testing for urobilin is by exam-

ining the urine directly by means of a small pocket spectroscope. If the urine is deeply colored it may be necessary to dilute it before the absorption spectrum can be seen. The characteristic band is one between the green and the blue parts of the spectrum, between the lines *b* and *F*. All the above solutions can also be tested, but if zinc chloride and ammonia have been added the spectrum extends more towards the green, the edge covering the line *b*. (See Fig. 152.) The chromogen, urobilinogen, does not give any absorption bands.

The absorption bands are sharper if a few drops of tincture of iodine are added to 10 c.c. of the urine.

**Uroerythrin.**—This pigment gives the pink color to urate deposits. It is present in the urine in extremely small quantities. It is of no practical interest, its chief characteristics being its great affinity for uric acid compounds, the ease with which its solutions are bleached by diffuse daylight, and its color reactions with the alkalies and acids. If a little of the pink urate precipitate is collected on a filter, dried, and touched with a drop of sodium hydrate solution, a bright green coloration is produced. The spectrum, examined preferably in solution in amyl alcohol, shows two ill-defined bands. The one nearer the red end of the spectrum is in the green between *D* and *E*, the other about the same place as the urobilin band.

**Melanin.**—The pigment derived from the cells of melanotic tumors occasionally appears in the urine and imparts to that fluid a dark brown color. The color deepens on exposure to the air or when an oxidizing agent, such as bromine water or ferric chloride, is added to the urine.

## BILE PIGMENTS

**Bilirubin.**—Bile pigments appear in the urine as such or in the form of a soluble combination of bilirubin with alkalies or the alkaline phosphates. In infants with icterus, however, the bilirubin is frequently in the form of crystals or masses, due to a low phosphate content of the urine. If the urine is allowed to stand exposed to the air the bilirubin becomes oxidized to the green biliverdin. Most of the tests for bile pigments in the urine depend upon this reaction.

The urine contains bilirubin when there is obstruction to the free outflow of the bile from the liver. The obstruction may take place in the smaller biliary capillaries, or in the larger ducts, or, finally, in the common duct. A jaundice due to alterations in the blood, a so-called hæmatogenous jaundice, is as yet unproven. The conditions in which bile may be expected in the urine are certain of the infectious diseases, in acute yellow atrophy, in cirrhosis, especially the hypertrophic form, in acute or chronic cholangitis, and in the simple catarrhal jaundice associated with gastro-intestinal symptoms. Among the mechanical causes for jaundice may be mentioned stone in the common duct, tumors or tuberculous lymph nodes pressing on the duct, and also tumors of the pancreas. The bilirubin of the bile is produced by the breaking down of the hæmoglobin of the red cells, and it is usually considered as identical with the pigment of old blood clots—hæmatoidin.

**Tests for Bilirubin.**—One of the simplest means of judging of the presence of bile in the urine is the presence of a dark yellow shade in such urine, with a faint green tint when the urine is viewed by reflected light. The foam of the urine is also a bright yellow color and is a valuable evidence of the presence of bile, although rarely a urine may be seen in which a large amount of urobilin is present giving a brownish yellow color to the foam. Only chemical or spectroscopic tests will allow of a differentiation of the two substances under these conditions. The very large amount of urobilin necessary to produce the yellow foam is usually present in urines from cases of liver disease, especially cirrhosis, in which true icterus alternates with this so-called urobilin icterus. When large quantities of bilirubin are excreted, crystals of this substance can be obtained by shaking out the urine with chloroform and allowing the latter to evaporate in a porcelain dish. Rhombic crystals of bilirubin are obtained which give the color reactions microscopically when nitric acid is allowed to run in under the cover glass. Chrysophanic acid, which appears in the urine after the administration of rhubarb or senna, and also santonin, often give the urine a dark color with a yellow foam, but the addition of the sodium carbonate used in Hammarsten's test gives rise to a pink or purple color and thus distinguishes the drug reactions. The color changes obtained by the addition of acid are yellow and do not show the play of tints characteristic of bilirubin.

**Gmelin's Test** for bilirubin is made by carefully layering the urine over strong nitric acid which contains a very small quantity of the fuming acid. Near the point of contact a green ring will be seen if the urine contain bilirubin. Below the green ring is a blue, and below that a red one; only the green ring is characteristic, the others may come from indoxyl or urobilin. The urine should be acid or rendered so with acetic acid, as otherwise bubbles of gas will rise through the fluid and break up the ring. This will also occur if pure fuming nitric acid is employed, and therefore the fuming acid should be diluted with ordinary strong nitric acid for use.

**Rosenbach's filtration method**[1] is based upon the fact that if a large quantity of urine containing bile is filtered through white filter paper, the bilirubin will be retained in the paper; and if a drop of nitric acid or 5 per cent. chromic acid be placed on the paper on its inner surface, a green spot will be produced, changing to red, or if chromic acid be used, only a green without any further color change. It is not a very reliable test.

**Smith-Rosin Test.**[2]—A mixture of alcohol and either Gram's solution or tincture of iodine, in such proportions that the mixture will be of a strong yellow color, is layered over the suspected urine. If bilirubin is present a green ring will be formed at the point of contact, from the alteration of the bilirubin. This test is one of the best for clinical purposes, but is not very sensitive, indicating 1 milligram in 10 c.c. or one part in ten thousand. Antipyrin may also give a green ring.

**Hammarsten's Method.**[3]—The reagent consists of a mixture of one volume of nitric and nineteen volumes of hydrochloric acid, each of a strength of about 25 per cent. This will keep for about a year. When one volume of the acid is mixed with four volumes of alcohol and a drop or so of bilirubin solution or urine is added to a few cubic centimeters of the reagent, the whole takes on a beautiful green color. If more acid be added gradually, the mixture changes through the whole scale of the Gmelin's reaction from a progressive oxidation of the bilirubin through the stages of green, which is biliverdin, blue, which is bilicyanin, to finally a red pigment, bilipurpurin, which changes to a yellow choletelin. This method of Hammarsten is useful in testing urine containing large quantities of urobilin and indoxyl, which ordinarily interfere with the color reactions, and also for very small quantities of bilirubin. In order to isolate the bile pigment, the urine, which should be rendered slightly alkaline with sodium carbonate, is precipitated with a small amount of barium, or better, calcium chloride, and the mixture centrifuged and the supernatant fluid poured off. A few cubic centimeters of the reagent are then poured over the precipitate and the whole again centrifuged. The fluid above the precipitate will be either green or bluish, and by a gradual addition of the reagent can be carried through all the stages of the Gmelin scale. The green color may be obtained when the urine contains not more than one part of bilirubin to 500,000 of the urine. If the amount of bile pigment be very small the fluid should be neutral and the proportions of the acids one to ninety-nine instead of one to nineteen. Or instead, one may use the same acid and dilute it with nine instead of four parts of alcohol. The supernatant fluid which is obtained after centrifuging can be used for the indoxyl test, as the presence of a large amount of bile often interferes with the direct examination of the untreated urine.

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[1] Cent. f. d. med. Wissen., 1876, p. 5.

[2] *Smith:* Dub. Med. Journal, 1876; *Rosin:* Berl. klin. Woch., 1893, p. 106.

[3] Abstract in Jahrb. f. Thier-Chemie, 1898, p. 310.

**Jolles' Method.**—Jolles [1] has modified his original method for the detection of bile pigment in the urine and recommends the following as an exceedingly delicate reaction, demonstrating less than one-tenth of a milligram of bilirubin. About 10 c.c. of the urine are mixed in a centrifuge tube with 3 c.c. of chloroform and 1 c.c. of a 10 per cent. barium chloride solution, and thoroughly shaken. The contents of the tube are then centrifuged and the fluid which remains above the chloroform and the precipitate poured off. The centrifuge tube is then filled with distilled water and the contents again centrifuged. Dark-colored urines may require a second or third treatment with distilled water. The chloroform and precipitated residue is then mixed with 5 c.c. of alcohol, thoroughly shaken, and two or three drops of an iodine solution added, and the whole filtered into a test tube. The iodine solution is made by dissolving 0.63 of a gram of iodine, and 0.75 of a gram of mercuric chloride, each in a separate 125 c.c. of alcohol. The two alcoholic solutions are combined and 250 c.c. of concentrated hydrochloric acid are added. The solution should be kept in a brown bottle, and is quite permanent. If bile pigment be present in the slightest traces in the urine, a characteristic green color is produced in the fluid after the addition of the iodine solution and, if necessary, standing a few minutes. The reaction can be hastened by slight warming on the water bath. In highly concentrated urines the test can be modified as follows: The residue which has been mixed with 5 c.c. of alcohol is poured from the centrifuge tube into a test tube, a couple of drops of the iodine solution are added, and the whole warmed for two or three minutes to about 70° C. on a water bath, and then filtered. The slightest traces of bile pigment are detected by the greenish or greenish-blue color of the filtrate. The reaction is not interfered with by either indoxyl, hæmoglobin, or methæmoglobin.

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[1] Deut. Archiv. f. klin. Medicin, Bd. lxxviii, 1903, p. 137.

**Bilicyanin** (*Cholecyanin*).—The blue color produced by the action of oxidizing agents on bilirubin is bilicyanin, which is of interest because its absorption spectrum is characteristic and affords another means of identifying the bile pigments. In acid solutions, such as are obtained by Hammarsten's test, two bands are visible between *C* and *E*, separated from each other by a narrow space about corresponding to *D*. Alkaline bilicyanin can be demonstrated by Stokvis'[1] test for bile pigment. The urine is precipitated with zinc acetate, and the precipitated zinc bilirubinate collected on a filter and washed with water. It is then dissolved in a small quantity of ammonia. The solution becomes brown from oxidation by the oxygen of the air and gives the cholecyanin spectrum in an alkaline solution. This consists of one sharp band between *C* and *D*, a faint band covering *D* and a very faint shadow between *D* and *E*. (See Fig. 152.) This test is not very sensitive and has not come into general use for clinical purposes.

## HÆMATURIA AND HÆMOGLOBINURIA

If the urine contains blood in small quantities the color of the fluid is not in any way altered; larger quantities cause more or less of a cloud to appear in the urine and a reddish sediment to collect at the bottom if the hæmorrhage be recent. If, however, the blood has remained for any length of time in the bladder, and especially if ammoniacal fermentation has taken place, the urine becomes more or less of a dirty brownish red, or even dark brown with a shade of green. The source of the bleeding may be anywhere along the genito-urinary tract, but certain differences which become of clinical value may be observed in blood from different regions.

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[1] Cent. f. d. med. Wissen., 1872, p. 785.

*a.* The blood in hæmorrhages from the kidney is thoroughly mixed with the urine, which remains acid unless there is also a pyelitis or cystitis. There are never any large clots in the sediment, but only casts of the kidney tubules, either composed entirely of blood corpuscles or of fibrin. As long-continued hæmorrhages from the kidney are usually connected with an acute nephritis, one would also expect casts and epithelium characteristic of such a condition. In hæmorrhages from the pelvis of the kidney, casts are often formed which more or less reproduce the shape of the pelvis. The bleeding from this site is due either to sharp stones, to small varicose vessels in the pelvis, or to inflammation. In the latter condition the urine will also contain pus.

*b.* Blood from the ureters, if the amount is considerable, is often passed in the form of cylindrical clots exactly reproducing the lumen. If the quantity is small no such diagnostic point exists, but the site of the bleeding may be determined by the cystoscope. Calculi and new growths are the most frequent causes of hæmorrhage.

*c.* Hæmorrhage from the bladder may be due to varicosities of the prostatic portion, to tumors, to stones, to tuberculous or other inflammation, or to parasites. A differential diagnosis can often be made by the examination of the urine. Small fragments of tumor tissue can sometimes be found in the sediment, which are not too necrotic to permit of a section being made and the morphology determined. The embryos of Filaria sanguinis or the eggs of Distomum hæmatobium can be easily identified. Tubercle bacilli should also be looked for, taking care not to be misled into a positive diagnosis by the almost constant presence of the smegma bacillus. The site of the lesion can best be determined by the cystoscope.

*d.* Hæmorrhage from the prostatic or penile urethra can best be localized by the use of two glasses into which the patient passes the urine. If the blood comes from the prostatic portion it will appear only at the end of micturition and not mixed with the first portions of the urine; the reverse is true of blood from the penile portion, which appears in the first glass and is washed out from the urethra, the last portions being clear.

*e*. Blood is usually present in the urine during menstruation, but it is important to remember that the blood may also be derived from a myomatous or carcinomatous uterus.

### HÆMOGLOBINURIA

In hæmoglobinuria the urine contains either hæmoglobin, methæmoglobin, or, very rarely, crystals of hæmatoidin. Red blood corpuscles are either entirely absent or in very small numbers. The urine is diminished in quantity, of a dark red color, and shows a dark red or black, finely granular sediment on standing. It may contain only traces of albumin.

Hæmoglobinuria or methæmoglobinuria is seen in tropical malaria, scarlatina, typhus, and syphilis. The phenomenon follows poisoning by the chlorates, naphthol, nitrobenzol, pyrogallic acid, sulphonal, antipyrin, phenacetin, or arseniuretted hydrogen —in fact, by any one of the substances which act destructively on the blood corpuscles. It has been seen after the eating of fresh mushrooms.

Hæmoglobinuria is also seen after the action of extreme heat or cold, and especially in that peculiar condition called paroxysmal hæmoglobinuria where overexertion or a slight chilling of the body is capable of inciting an attack. It has been shown experimentally that the hæmoglobin or the methæmoglobin can be detected in the circulation before its excretion by the kidneys; in fact, if only a small quantity is set free it may not appear in the urine, but is destroyed by the liver, spleen, and bone marrow.

A few cases of cyanosis with methæmoglobinæmia have to be described, occurring in persons without such lesions of the heart or lungs as usually accompany this condition. The cyanosis appears to be due to the formation of methæmoglobin in the blood by the action of toxic agents. The spectrum of the methæmoglobin in alkaline solution can occasionally be demonstrated in the blood of such cases by viewing the skin of the hand through a spectroscope, the surface being intensely illuminated either by sun or electric light. Stokvis [1] describes a case which he considers due to a severe intestinal disorder. The urine of this case had the power of converting hæmoglobin to methæmoglobin in a very short time and contained considerable quantities of indol and skatol compounds. In Talma's [1] cases also the urine contained large quantities of indol and skatol. The methæmoglobin was confined to the red cells and not diffused through the serum, as could be shown by allowing the blood to clot in a cool place and examining the separated serum with the spectroscope.

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[1] *Stokvis:* Zur Casuistik d. autotoxischen Cyanosen. Festschrift v. Leyden, Bd. i, 1902, p. 597 (Literature).

**Tests for Blood in the Urine.—Microscopic.**—The simplest method of determining the presence of blood in the urine is to examine the sediment microscopically, which is obtained preferably by the centrifuge, as the red corpuscles are altered by prolonged action of the urine. The red corpuscles may be recognized as small circular disks, which are more regular in form than any other morphological element found in the urine, though rarely small spheres of calcium oxalate may closely imitate the appearance of the red cell. If the urine has stood any length of time the corpuscles may be swollen and difficult to identify, or they may have shrunken and become crenated. In alkaline urine it may be difficult to find anything but faint shadows of the red cells, all the coloring matter having been dissolved out. In such case it is necessary to have recourse to other tests. In hæmorrhage from the kidney parenchyma, the blood may be found in the form of cylindrical casts of the tubules.

**Guaiacum Test.**—Shake up in a test tube equal volumes of a fresh 1 per cent. alcoholic tincture of guaiacum and of turpentine which has become oxidized by long exposure to the air and light. The urine to be tested is layered under this mixture. If blood or blood pigments are present there is formed at the point of contact first a bluish-green and then a brilliant blue contact ring. Normal or albuminous urine does not give this reaction, but it is given by urine containing pus; but the color produced by pus disappears on boiling the mixture, while that caused by blood does not. Alkaline urine is first to be made acid before testing. In case of doubt the urine should be shaken out with ether after acidulating with acetic acid. For details see pages 16 and 329.

**Heller's Test.**—Heat the urine to boiling and add some sodium hydrate solution, and then heat again for a short time. If blood be present the phosphate precipitate brought down by the heat and alkali will be colored red by the precipitated hæmatin, the hæmoglobin being broken up by the action of the heat into a coagulated histon and hæmatin. If the reaction of the urine be alkaline to begin with, the phosphates will probably be already precipitated, so that it is necessary to add an equal bulk of normal acid urine, which will supply the needed phosphates. The precipitate may be collected on a filter, the phosphates dissolved by a little acetic acid, and the remainder, which consists of hæmatin, tested by Teichmann's test for hæmin crystals, or dissolved in alcohol acidulated with sulphuric acid and examined with a spectroscope for the bands of hæmatin.

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[1] *Talma:* Intraglobular Methæmoglobinæmia. Berl. klin. Woch., 1902, p. 865.

**Spectroscopic Test.**—This is the most rapid and the most satisfactory method for the detection of blood in the urine. The urine is placed in a test tube or small trough with plate-glass sides and examined with a small direct-vision spectroscope. It is well to pour some water on the surface of the urine, but not to mix the two, and thus obtain a mixture of graduated intensity from the pure water on the top to the pure urine at the bottom. At some point the absorption bands will show, if the urine contain blood.

The spectrum may show the two bands of oxyhæmoglobin, the single band of reduced hæmoglobin, or the four-banded spectrum of methæmoglobin. The latter is to be recognized in the presence of the other two by its dark band in the red between *C* and *D*, if the reaction of the urine be acid.

Very rarely the urine may show the spectra of hæmatin. In the untreated urine the spectrum can scarcely be distinguished from that of methæmoglobin; but if the urine be rendered strongly alkaline by means of ammonium hydrate the band in the red corresponding to *C* will shift to a diffuse band to the left of *D*, and then, if ammonium sulphide be added, the spectrum of reduced hæmatin or hæmochromogen slowly appears with two bands between *D* and *E* like oxyhæmoglobin, only a little nearer the green. (See Fig. 2, p. 13.)

The delicacy of the spectroscopic detection of blood in urine, as well as in other fluids, is much increased by the use of a method suggested by Donogány,[1] which depends upon the formation of hæmochromogen. In urine the process is as follows: To 10 c.c. of urine add 1 c.c. of ammonium sulphide and 1 c.c. of pyridin.

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[1] Virchow's Archiv, Bd. cxlviii, 1897, p. 234.

If the urine contain blood the fluid assumes a more or less intense orange red color. This may be only visible, when the blood is in small quantity, on looking through the test tube lengthwise so as to obtain a thicker layer of fluid. If the urine is deeply colored with other pigments the spectroscope must be used to verify the result. The spectrum of hæmochromogen consists of two bands in the yellow green between *D* and *E*, but nearer to *E* than the second line of the oxyhæmoglobin spectrum. The advantage of the method depends upon the much greater visibility of the reduced hæmatin spectrum over that of hæmatin or hæmoglobin, as it will show one part of blood to eight thousand of urine; while the oxyhæmoglobin spectrum is rarely visible unless more than one part of blood in two thousand is present. In testing stomach contents or fæces the blood pigment should be dissolved out by 20 per cent. sodium hydrate, the pyridin added, and if necessary some ammonium sulphide. It is well to filter the mixture in order to see the orange-red color. The reaction can be used microscopically by allowing a drop of the suspected fluid, which has been mixed with an equal quantity of pyridin and 20 per cent. sodium hydrate to dry on a slide. After several hours brownish hæmochromogen crystals will separate out in the form of radiating needles.

### HÆMATOPORPHYRINURIA

Hæmatoporphyrin is an iron free derivative of hæmoglobin and is present in traces in normal urine. It is occasionally found in the urine in cases of paroxysmal hæmoglobinuria,[1] and in some diseases, such as rheumatism, phthisis, Addison's disease, exophthalmic goitre, cirrhosis of the liver, and lead-poisoning, but not constantly. The pigment is also occasionally seen after internal hæmorrhages.

In chronic poisoning, from the long-continued use of sulphonal and trional, the presence of hæmatoporphyrin is quite frequent and of diagnostic value. It has also been obtained from the blood in a fatal case.[2] The pigment can not usually be detected in the urine by the spectroscope. To isolate it from the urine, 50 c.c.

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[1] *Pal:* Cent. f. inner. Med., 1903, p. 601.

[2] *Taylor* and *Sailer:* Contributions from the Pepper Laboratory, Philadelphia, 1900, p. 120.

are precipitated with a mixture of a 10 per cent. solution of barium chloride which has been saturated with barium hydrate. The precipitate is washed first with water and then with a little strong alcohol. The damp mass is then scraped off the filter paper and shaken up with 1 c.c. of hydrochloric acid and about 10 c.c. of strong alcohol. Filter off the alcoholic extract through a dry filter paper and examine it with a spectroscope. If hæmatoporphyrin be present the two bands in the spectrum at *D* and near *E* can be seen; if now the mixture be rendered alkaline with ammonia, the four absorption bands of alkaline hæmatoporphyrin will become visible.

Another method which is applicable to urines containing a considerable amount of phosphates is to precipitate these salts by adding 20 c.c. of 10 per cent. sodium hydrate to 100 c.c. of urine. The precipitate is freed from an excess of water by pressing between layers of filter paper and then shaking up in a test tube with a mixture of 1 c.c. concentrated HCl and 10 c.c. of alcohol, and the extract examined for the two-banded spectrum.

In those cases in which the spectrum of the pigment can be made out directly in the urine without preliminary treatment, the spectrum seen, as a rule, is the four-banded alkaline one, even though the urine be normally acid in reaction; but rarely a two-banded spectrum is seen, which on the addition of a little acid alters slightly, the bands moving slightly apart, or finally changes to the acid hæmatoporphyrin spectrum. (See Fig. 4, page 16.) This two-banded spectrum is frequently seen when examining the pigment carried down by the urates.

## CHOLESTERIN

This substance is a monatomic alcohol normally present in the nerve tissues, bile, etc. It does not appear in the urine in health, but occasionally is found where there is an extensive fatty degeneration of the kidney, during the course of a nephritis, or after the rupture of old abscesses into some portion of the genito-urinary tract, and finally, in cases of chyluria. The crystals are colorless, rhombic, flat plates, usually with a notch at the acute angle. (See Fig. 103, p. 361.)

The tests for cholesterin are given on page 338.

## BILE ACIDS

The bile acids which appear in human urine are taurocholic and glycocholic acids. They exist as sodium salts in the urine. They appear in traces in the urine in biliary obstruction with jaundice; but, as a rule, their excretion is in much smaller quantity than would be expected from the amount produced by the liver. For example, in extreme icterus only 3 to 4 centigrams of bile acids have been obtained when 8 to 12 grams were produced in the liver in twenty-four hours. It is possible, however, that in severe icterus due to acute atrophy of the liver substance the production of bile acids is diminished, and the small quantities which appear in the urine under these conditions are thus explained.

The older writers separated jaundice into two types, hepatogenous and hæmatogenous, and stated that the bile acids were present in the urine only in the hepatogenous icterus. Recent investigations of the pathology of icterus have shown that this condition is practically always due to anatomical changes in the liver and is therefore in all cases hepatogenous, so that the fact that bile acids are found in only about 25 per cent. of the cases of jaundice can not be explained on this basis.

A simple and reliable test for bile acids does not exist. **Hay's test** is dependent upon the reduction of surface tension of the urine due to the presence of bile acids. This physical alteration in the properties of the urine may be shown by dusting flour of sulphur over the surface of the specimen of urine. If the sulphur sank, the author claimed that bile acids were present; if the sulphur floated, that the urine was free from these bodies. The test, however, is not reliable.

**Pettenkofer's Test.**—As it is impossible to recognize bile acids in the urine by any tests which can be applied directly, it is necessary to isolate these acids from a considerable quantity of the urine, and the following method is the best: Two hundred c.c. of urine are freed from albumin by acidulation and boiling, and then precipitated with lead subacetate and a small quantity of ammonia. The bile acids combine with the lead to form a lead salt and the precipitate which forms contains them, though contaminated with a large number of other substances. This precipitate is washed with water on a filter, and after drying is extracted several times with boiling absolute alcohol and the alcohol filtered off while still hot. The alcoholic solution of the taurocholate and the glycocholate of lead thus obtained is evaporated to dryness with a few drops of sodium carbonate solution. The residue is again extracted with hot absolute alcohol, and the resulting solution is concentrated to a small bulk. A large quantity of ether is then poured over the alcoholic residue, by which means the bile acids are precipitated, as they are not soluble in ether. The crystals are collected on a filter and dissolved in water. A few c.c. of the aqueous solution of the crystals are mixed with several drops of a 10 per cent. solution of cane sugar and with a little concentrated sulphuric acid. The optimum temperature for the reaction is about 70° C., and if the bile acids are present, a red color is first produced and then a beautiful violet. The violet solution should be diluted with water and examined spectroscopically. A band between *D* and *E* and a second band to the red side of *F* is given by the bile acids. (See Fig. 152, page 561.)

Instead of cane sugar, a one to one thousand solution of furfurol may be used. After the addition of sulphuric acid a bright cherry red color is produced if cholic acid be present.

The same method as given above furnishes approximately quantitative results when carried out on a large amount of urine. The resulting alcoholic solution is decolorized with animal charcoal and examined in a polariscope. The specific rotation of glycocholate and taurocholate of soda in alcohol is + 25.7 and +24.5, respectively.

## SUCCINIC ACID

This substance is a constituent of normal urine, and is probably derived entirely from the food. An increased amount has been noticed after the drinking of large quantities of wines which contain traces of this substance, and also after the eating of asparagus. Its presence in the urine is of no importance from a clinical point of view.

## OXALURIC ACID

This substance is present in traces in normal urine as ammonium oxalurate. This acid is derived from the oxidation of uric acid in the body. No points of clinical interest are connected with its presence in the urine.

## LACTIC ACID

In normal urines lactic acid is not present. The inactive lactic acid is occasionally found in diabetic urine as a result ot fermentation of the sugars present. The dextrorotatory lactic acid derived from muscle is found in the urine in acute phosphorus and carbon monoxide poisoning, in acute yellow atrophy of the liver, occasionally in cirrhosis or other severe hepatic lesions, and recently in the urine of persons suffering from epilepsy, but only after an attack. Prolonged and severe bodily exertion may also cause lactic acid to appear in the urine.[1]

**Tests.**—The urine is evaporated on a water bath and the residue is rapidly extracted with warm 95 per cent. alcohol. The alcohol is evaporated and the residue strongly acidulated with phosphoric acid and extracted with ether. The residue remaining after the distillation of the ether is neutralized with barium hydroxide. The solution is filtered, concentrated to a small volume, and the barium salt precipitated by alcohol. The barium lactate is collected, washed with absolute alcohol, dried, digested with phosphoric acid, and again shaken out in ether. The lactic acid which remains after evaporation of the ether is treated with zinc carbonate, and the filtrate from the excess of zinc is set aside and allowed to evaporate spontaneously. The crystals of zinc lactate are small, blunt prisms, which are soluble in water and alcohol and are lævorotatory. They contain 12.9 per cent. of water crystallization, which escapes at a temperature of 105° C. Some of the crystals should be washed with a small amount of absolute alcohol, and then carefully dried at room temperatures. A weighed amount is then heated for about three hours at a temperature of 105° C., when the salt should lose 12.9 per cent. of its weight.

These reactions are usually sufficient to characterize the salt.

## FAT IN THE URINE

Small amounts of fat may appear in the urine in the form of globules in a variety of conditions. Among these are fractures of the long bones with the formation of fat emboli, eclampsia, diabetes, pulmonary tuberculosis, phosphorus poisoning, nephritis, and chronic gastritis. Moderate quantities of fat in droplet form may be found in the urine after large doses of cod liver oil. Fat is also found in the urine in a finely divided form, derived from one of the lymphatic vessels in the bladder walls becoming filled with the embryos or mature worms of the Filaria sanguinis and rupturing, thus allowing the escape of the chyle from the vessel into the urine. The fat in this condition is in such small drops as to be with difficulty demonstrated under the microscope. At times this condition of chyluria appears in those who are not infected with the Filaria and in whom no definite reason can be found for the presence of the fat. The presence of Bilharzia embryos may also incite chyluria.

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[1] *Inouye* u. *Saiki:* Zeit. f. phys. Chemie, Bd. xxxvii, 1903, p. 203; *Colasanti* u. *Moscatelli:* Arch, f. exp. Path. u. Pharm., Bd. xxvii, p. 158.

Care should be taken not to be misled in the microscopical examination of the urine by particles of fat derived from the lubricant of a catheter or fatty material left in the vagina after an examination and washed into the specimen by the stream of urine, and also to remember that hysterical persons and malingerers occasionally pour milk into the urine for purposes of deception. The presence of lactose in the urine will furnish a clue to the source of the chyluria.

The presence of fat may be determined by shaking the urine with ether after having rendered it strongly alkaline. If the fluid contains fat it will clear considerably, but a slight opalescence always remains, due possibly to cellular débris not dissolved by alkali. The urine may be mixed with osmic acid or Sudan III, and the black or red fat globules studied microscopically.

## THE VOLATILE FATTY ACIDS IN THE URINE[1]

Traces of volatile fatty acids are present in all normal urines. They are formed, in all probability, in the intestinal tract by bacterial action on the food, and after absorption undergo oxidation in the body. A small portion, however, escapes in the urine. These acids are usually increased in febrile conditions, though exceptions may be observed, as, for example, low values, that is, from 30 to 50 equivalents of $^{N}/_{10}$ acid, are to be seen in scarlatina, erysipelas, diphtheria, and pneumonia before the crisis. On the other hand, in all septic conditions where there is breaking down of tissues with resorption of the products of bacterial activity, the volatile acids are increased; especially is this the case in purulent or bloody exudates and in suppurative processes in the lung. After the beginning of convalescence in pneumonia, large quantities of acids are excreted owing to the resorption of the pneumonic exudate. Values as high as 240 c.c. of $^{N}/_{10}$ acid may be observed. High values are seen in cases of gastrectasia and ulcer of the stomach, where the secretion of hydrochloric acid is high or normal. When there is a subnormal secretion of acid in the stomach the fatty acids in the urine are low. The clinical value of the determination of the fatty acids is so slight that the technique may be omitted.

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[1] *F. Rosenfeld:* Deut. med. Woch., 1903, p. 224.

## FERMENTS IN URINE

**Pepsin.**—The fact that traces of pepsin are present in the urine has been known for many years, but the phenomenon has no diagnostic value. The demonstration of the ferment may be made by adding dilute hydrochloric acid and a few fragments of fibrin to a specimen of urine and keeping the mixture at incubator temperature for a few hours.

The presence of pepsin in the urine has given rise to some of the unwarranted conclusions which have been drawn concerning the frequent occurrence of peptone in urine in which albumose is present, the error being due to the fact that the latter substance is altered into peptone after the urine is passed.

**Lipase.**—This ferment is not present in normal urine, but in several cases of hæmorrhagic pancreatitis with fat necrosis, it has been possible to demonstrate the presence of a fat-splitting ferment.[1] As yet the test has not been carried out on a sufficient number of cases to warrant the drawing of diagnostic conclusions from a positive result. The technique is as follows: [2]

The urine is divided into two portions and neutralized with sodium hydroxide. One specimen is boiled to destroy the ferment which may be present. To each specimen a few drops of ethyl butyrate and a small quantity of toluol are added. Both specimens of urine are then kept at 37° C. for an hour. If a fat-splitting ferment is present the ethyl butyrate will be split with the setting free of butyric acid and the production of a well-marked acid reaction in the solution. The boiled specimen, which is used as a control, will remain approximately neutral. The amount of ethyl butyrate split up may be estimated by shaking out the specimen with neutral ether and titrating with $^N/_{10}$ sodium hydrate after evaporation and solution of the residue in distilled water.

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[1] *Opie:* Diseases of the Pancreas, Phila., 1903; also Johns Hopkins Hosp. Bull., vol. xviii, 1902, p. 117; *Hewlett:* Jour. Med. Research, vol. xi (N. S., vol. vi), 1904, p. 377.

[2] *Kastle* and *Loevenhart:* Amer. Chemical Jour., 1900, vol. xxiv, p. 491.

## DRUG REACTIONS

In the examination of the urine it is often important to be able to determine the presence or absence of certain drugs administered as medicine or taken as poisons. Facts of importance may often be ascertained as to the absorption of drugs by suitable tests, as for example in iodoform or carbolic acid poisoning due to absorption from dressings.

**Acetanilid** (*Antifebrin*).—This substance appears in the urine partially as an ethereal sulphate, partly in conjugation with glycuronic acid. The urine is lævorotatory, often reduces Fehling's, and may contain considerable quantities of urobilin. Occasionally the urine assumes a dark green color. Acetanilid gives the indophenol reaction, as follows:

Ten c.c. of urine are boiled with 2 c.c. of concentrated hydrochloric acid. After cooling, several cubic centimeters of 3 per cent. aqueous carbolic acid are added and a little chromic acid or 1 per cent. calcium hypochlorite solution. If acetanilid be present, the fluid becomes red, and if rendered alkaline with ammonia, a strong blue color is developed. Phenacetin gives the same reaction, but may be distinguished by the following test: Add to the urine two drops of a 1 per cent. sodium nitrite solution, a few drops of sodium hydrate, and a little alkaline solution of *a*-naphthol. If phenacetin be present, a red color will appear which changes on the addition of hydrochloric acid to a violet.

Dark-colored urines should be concentrated by evaporation, boiled for a few minutes with hydrochloric acid, and, after cooling, shaken out with ether. The residue should be dissolved in water and the test carried out in aqueous solution.

**Antipyrin.**—This substance is excreted partly as such in the urine, partly as oxyantipyrin glycuronic acid. According to Penzoldt, urine containing considerable quantities of antipyrin may show a deep red color and be dichroic, the solution being red by transmitted light, and green by reflected light. Occasionally albuminuria, glycosuria, and hæmaturia have been noted, the latter being due to the hæmolytic action of the drug on the blood.

*Tests.*—1. The addition of a couple of drops of ferric chloride produces a dark red color, which does not disappear on boiling the solution. This distinguishes the substance from diacetic acid. Dark-colored urines should be rendered alkaline with ammonia and shaken out with chloroform and the residue obtained after evaporation of the chloroform tested with ferric chloride.

2. The urine is boiled with hydrochloric acid and after being neutralized with sodium hydrate is distilled, when antipyrin can be detected in the distillate by the greenish color which is produced by the addition of sodium nitrite and acetic acid. A green ring is given by urine containing antipyrin with the Smith-Rosin bile test.

**Aristol** (*dithymoldiiodid*).—Iodine can be detected in urine. (See also under thymol.)

**Asaprol.**—This substance gives a blue color with ferric chloride.

**Arsenic** in urine. See p. 709.

**Atropine** is excreted as such in the urine. The tests are:

1. A few drops of the urine are placed in the conjunctival sac of a cat's or rabbit's eye, and cause permanent dilatation of the pupil.

2. The residue obtained by evaporating the urine on a water bath after the addition of a couple of drops of strong nitric acid, gives a violet changing to a red color when mixed with some alcoholic potash.

**Betol.**—Salicylic acid can be demonstrated by the addition of ferric chloride.

**Bromine.**—Bromine or a bromide can be detected by adding a little fresh chlorine water to the urine and shaking out with chloroform. The latter will be colored yellow. The reaction is difficult to obtain if only traces of bromine are present, in which case it is better to employ another method[1] which furnishes much more accurate and definite results, but is somewhat complicated.

Ten to twenty c.c. of urine are rendered alkaline with sodium carbonate and evaporated to dryness in a platinum crucible, and then strongly heated to char the residue. The blackened mass is extracted with a few c.c. of distilled water, acidulated with hydrochloric acid, some chlorine water is added, and the mixture shaken out with carbon bisulphide. If bromine is present a yellow color will be produced in the bisulphide. If both iodine and bromine are present, the blue of the iodine masks the yellow color. If iodine is present only in traces, it is possible to demonstrate the bromine by adding more chlorine water and shaking, but this often fails. It is possible in this dilemma to use petroleum benzine to separate the two substances.[2] If about 1 c.c. of this substance be added to the test tube containing the bisulphide extract which has been decolorized by adding chlorine water, the iodine will be extracted in the benzine and float on the surface of the fluid as a faint pink layer.

**Brucine** is excreted unchanged in the urine. The evaporated residue gives a blood red color with concentrated nitric acid, and the same with concentrated sulphuric acid plus potassium nitrate.

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[1] *Salkowski:* Zeit. f. phys. Chemie, Bd. xxxviii, 1903, p. 157.

[2] *Cathcart:* Zeit. f. phys. Chemie, Bd. xxxviii, 1903, p. 165.

**Chloral hydrate** appears in the urine almost wholly in the form of urochloralic acid, which is a glycuronic acid compound. The urine may reduce Fehling's after prolonged boiling, but does not always do so. It does not reduce Nylander's solution. Urochloralic acid can be separated by the addition of lead subacetate and decomposition of the precipitate by hydrogen sulphate. The presence of the glycuronic acid component can then be determined by tests for that substance.

**Chloroform** is almost wholly decomposed in the body, and according to Vitali [1] is not excreted as chloroform in the urine after narcosis, but such urine may occasionally contain bile pigment, albumin, casts, and blood, and also sugar. Chloroform reduces Fehling's solution.

**Chrysarobin.**—This substance is chiefly excreted as chrysophanic acid, for the test for which see Rhubarb.

**Codeine** in large doses gives a red color with Obermayer's reagent.

**Cacodylic Acid** $(CH_3)_2AsO_2H$.—The sodium salt of this substance is used therapeutically. A portion of the arsenic appears in the urine as arsenious acid, while a larger part of the cacodylic acid is excreted as such.

*Tests.*—The urine gives a marked odor of cacodyl, and when heated with phosphoric acid gives that of garlic. Arsenic can also be detected in the urine by the Marsh test for more than two months after stopping the drug. In using this test, however, it is important to remember that arsenic is often present in the urine in recognizable amounts in persons who have not taken it in drug form.

**Camphor.**—Camphor appears in the urine as camphor-glycuronic acid, which can be recognized by lævorotation and the tests for the glycuronic acid.

**Cantharidin.**—After internal use of large quantities of this substance, the urine may contain albumin, casts, and blood. The cantharidin can be recognized as such by boiling the urine with potassium hydrate, decomposing the potassium cantharidate with sulphuric acid and extracting with chloroform. The residue after evaporation of the chloroform can be dissolved in almond oil and tested on an animal to see if it possesses the power of blistering.

**Caffeine** may appear in the urine unchanged or may be transformed into xanthin. The xanthin may be isolated as is given under the determination of the xanthin bodies in the urine, and tested by the reactions for that substance. See pages 489 and 610.

**Cocaine** may be found in the urine, but is usually rapidly decomposed in the body.

*Tests.*—The tests which may be used are:

1. The solution heated with 2 or 3 c.c. of chlorine water and then two or three drops of 5 per cent. palladium chloride solution, will give a brilliant red precipitate.

2. One per cent. potassium permanganate solution added drop by

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[1] Bollet. chim. farm., Bd. xxxviii, 1899, p. 249.

drop to a watery concentrated cocaine hydrochloride solution gives rise to crystals of cocaine permanganate.

3. Cocaine may be excreted in the form of a conjugate compound with glycuronic acid.

**Colchicin** when present in the urine gives a violet solution; when evaporated with concentrated nitric acid the color changes to a brownish red, and finally on the addition of an alkali to an orange red.

**Copaiba** is excreted in the urine as a glycuronic acid compound of the terpene group.

1. On addition of hydrochloric acid a cloudiness is produced by the separation of the resinous acid, then a brilliant red color, becoming violet on heating. The reaction is hastened by the addition of oxidizing agents, such as chloride of lime or tincture of iodine. The hydrochloric acid solution shows three absorption bands, one in the orange, one in the green, and one in the blue.

2. A cloud is induced in the urine by the action of acids and heat, but disappears when shaken up with petroleum ether, which differentiates these substances from the albuminous bodies. The urine is lævorotatory and reduces Fehling's after prolonged boiling.

**Creolin.**—The urine contains an increased quantity of conjugate sulphates, while the preformed sulphates are diminished. The urine is dark colored from the presence of phenol and cresol.

**Cryofin.**—This substance gives the same reactions as phenacetin.

**Dionin** is a hydrochloride of ethyl morphine and gives the morphine reactions.

**Euphorin** and **exalgin** give the indophenol reaction, either directly in the urine or after distillation with potassium carbonate.

**Exodin** (*Diacetyl-rufigallic-acid-tetramethyl-ether*).—The urine is dark in color, but possesses no staining power as it does after the use of purgatin. The addition of an alkali does not change the color, and the ordinary reactions for blood and diazo-substances are not interfered with by the drug.

**Guaiacol** is excreted unchanged in the urine and must first be isolated before testing.

A small amount of hydrochloric acid is added to the urine and the mixture is distilled by conducting steam from another flask into the one containing the urine. The distillate is mixed with dilute ammonia and again distilled. The first portion of the distillate is dissolved in an equal volume of ether and an excess of concentrated alcoholic potash solution is added. The compound of potash with guaiacol is insoluble in ether and separates out. It can then be decomposed with sulphuric acid and the guaiacol set free dissolved in alcohol, and a trace of ferric chloride added which gives a blue color; further additions give a yellowish green.

**Heroin** gives some of the morphine reactions.

**Iodoform** after absorption can be detected in the urine as an organic iodine compound, for the tests for which see under Iodine.

**Iodine** appears very promptly in the urine after the administration of its compounds, especially of the alkaline salts. It is present in the form either of an albuminous compound or as iodohippuric acid. Excretion is less rapid with the organic compounds, such as iodipin, for in this special case the absorption of the iodine is necessarily preceded by the splitting of the iodipin by the intestinal juices.

*Reactions.*—Iodine can be detected in the urine by adding a few drops of strong nitric acid to set free the iodine from its combination, and then extracting the iodine with chloroform. If iodine is present, the chloroform takes on a beautiful pink, or, if the amount be large, a reddish violet color. The same reaction is often seen in carrying out the indican test with Obermayer's reagent when a large amount of indican is not present. In case the latter substance is abundant in the urine, the blue color will mask the pink of the iodine.

Another reaction which is characteristic is with starch. A few drops of starch paste are added to the urine and the latter layered over some strong nitric acid. A deep blue ring will appear at the point of contact of the two fluids if traces of iodine are present. The color soon fades, especially after heating.

Both these reactions indicate only relatively large quantities of the drug. When the amount is small, as is likely to be the case in iodoform absorption, the tests of Harnack [1] are preferable.

*a.* The urine is acidified with dilute sulphuric acid and one drop of fuming nitric acid and then shaken out with chloroform.

*b.* The urine is mixed with a little starch paste, some dilute sulphuric acid and some fuming nitric acid. A blue or black color is produced, depending upon the amount of iodine present. If the fluid be heated the color disappears and returns on cooling. Large quantities of urine must be employed if faint traces of iodine are to be detected; for example, in amounts of 1 milligram or less.[2] Traces of iodine may be detected by evaporating a liter or more of urine which has been rendered alkaline with sodium carbonate and carrying out the above tests on the concentrated residue.

**Kairin** appears in the urine as an ethereal sulphate, occasionally accompanied by methæmoglobin.

*Tests.*—On the addition of ferric chloride to urine containing kairin a deep violet color is produced, and after the addition of acetic acid and chlorate of lime solution, a fuchsin red color. Kairin urine is also lævorotatory, may reduce Fehling's on prolonged boiling, and contains glycuronic acid.

**Lactophenin** gives the indophenol reaction.

**Lead.**—The detection of traces of lead in the urine is often of the greatest importance in obscure cases of lead poisoning, especially in the cerebral type of the disease, where the usual clinical evidences of the intoxication may be absent. The amount of lead excreted may be either considerable, as in cases of acute poisoning, where one of the salts of the metal may have been taken either by accident or with intent to produce abortion, or the amount may be very minute, as in chronic poisoning from the use of paints or the drinking of fluids containing traces of lead. In the first set of cases the lead may occasionally be detected in the urine by a simple qualitative test, if it is present in *quantities greater than* 50 *milligrams to the liter,* though even when considerable amounts have been ingested the excretion in the urine may not exceed 1 milligram to the liter, the greater portion being carried off in the fæces.

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[1] Berl. klin. Woch., 1882, pp. 297 and 788.

[2] *E. Rogovin:* Zeit. f. phys. Chemie, Bd. xxxviii, 1903, p. 157.

For the detection of lead when present in considerable quantities the method of v. Jaksch[1] modified by Hill[2] may be used. A bright clean strip of chemically pure magnesium ribbon is placed in the fluid to be tested. Ammonium oxalate in the proportion of about 1 gram to 150 c.c. is added. If lead be present it will be deposited on the magnesium, often within an hour, though it is preferable to allow the process to continue for twenty-four hours. The strip is then washed in distilled water and dried. The gray deposit of lead on the surface of the magnesium may then be tested.

*a.* Warm the ribbon with a crystal of iodine on its surface, being sure to continue the heating long enough to volatilize any excess of the iodine, which may otherwise leave a yellow stain, but not raising the temperature sufficiently to cause the magnesium to ignite. If lead be present, yellow lead iodide will be formed.

*b.* Dissolve the deposit in nitric acid and add potassium bichromate to one portion, when a yellow precipitate of lead chromate will be formed.

*c.* Add some hydrogen sulphide water to another portion of the nitric acid solution, when a black precipitate of lead sulphide will be formed. In most cases of chronic lead poisoning this simple test *fails utterly* to detect the lead which is present in the urine, and errors have frequently been made by considering a slight yellow stain on the ribbon as evidence of lead, when it is only due to the iodine coloring the magnesium oxide. Possibly this may be due to the fact that the lead is in organic combination and is not therefore easily precipitated on the magnesium ribbon.

**Electrolytic Test.**—The most reliable method is the following:

A small quantity of lead in the urine can be determined by electrolytic methods, which are simpler and quicker than a chemical examination in which large quantities of urine are evaporated and the residue tested. A convenient modification of the electrical method has been recently devised.[3] Two hundred and fifty c.c. of the urine to be tested are placed in a porcelain dish and 2 c.c. of chemically pure sulphuric acid are added. The mixture is heated gently, and about 5 grams of potassium persulphate are added in small portions with occasional stirring. The fluid is gradually brought to the boiling point, and in about half an hour the contents of the dish will be almost colorless from the destruction of the organic matter. If the fluid blackens, it is necessary to continue the heating, with the addition of more acid in a Kjeldahl flask until the color is discharged.

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[1] Klin. Diagnostik, 1901, p. 238.

[2] Lancet, 1897, p. 164.

[3] *Jacob* and *Trotman:* Brit. Med. Journal, vol. i, 1903, p. 242.

The heating is continued until the liquid is concentrated to about 50 c.c., and it is then placed in a platinum dish and a current passed through it for about eight hours. The current may be obtained either from the street mains or from a storage battery, or from a couple of primary cells. Fig. 153 shows a simple arrangement by which the current can be obtained from the street mains.[1] The platinum dish should be the negative pole, which can be determined by introducing the terminals into a small quantity of sodium chloride solution containing a few drops of phenolphthalein; a pink color appears near the negative pole. After the current has been passed a sufficient time, the platinum dish is washed in distilled water, nitric acid is added, and after removing the excess of acid by heating, a stream of hydrogen sulphide is passed through the liquid. The quantity of lead present may be estimated by matching the color with a standard solution of lead nitrate through which hydrogen sulphide has been passed. Usually, in lead poisoning, the quantity of lead contained in a liter of urine is too small to be conveniently estimated by gravimetric methods. Care must be taken in the selection of lead-free chemicals and dishes, and a blank experiment should always be made with each fresh supply of material.

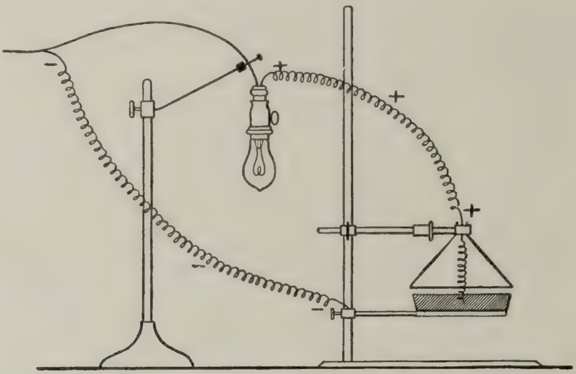

Fig. 153.—Diagram of Apparatus for the Electrolytic Determination of Lead, Using Current from the Mains.—(Jacob and Trotman.)

**Lysol** is a mixture of the cresols, and when ingested increases the amount of conjugate sulphates in the urine. It also gives the phenol reactions.

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[1] Usually about three lamps should be placed in series instead of the one shown in the figure.

**Mercury.**—The determination of the presence of this metal in the urine is chiefly of interest in connection with acute poisoning of a doubtful nature, or in order to estimate the amount of the substance absorbed after giving a patient inunctions of mercurial compounds. About 500 c.c. of the urine to be tested are acidulated with 50 c.c. of hydrochloric acid, a small ball of pure copper-gauze is then dropped into the fluid and the whole gently warmed for an hour. Instead of the gauze, a slip of copper-foil may be used. After warming, the fluid should stand for several hours, that all the mercury may be deposited on the gauze or foil. The gauze is then washed off with very dilute sodium hydrate solution, alcohol, and ether, to remove the sticky resinous deposit, which often gathers on the surface of the metal, and is dried. The whole or a portion of the copper is then heated in a long tube of infusible glass to volatilize the mercury, which, if present, collects in the upper portion of the tube and can be recognized by means of a magnifying glass as composed of fine shining globules. To complete the test the mercury can be converted into an iodide by gently heating a small crystal of iodine in the tube and allowing the vapor to pass over the deposit of metallic mercury. The yellow iodide is formed, which on standing, or more quickly by rubbing with a glass rod, is altered into the red form which can be more easily seen by the naked eye. One milligram of mercury to the liter of urine can be detected by this method.

A somewhat more rapid method has been suggested by Marshall,[1] as follows: One hundred c.c. of strong hydrochloric acid are added to 600 c.c. of urine together with one-half gram of solid potassium chlorate. The mixture is then heated to the boiling point in a porcelain dish and while hot transferred to a funnel provided with a stop-cock. The stop-cock is closed and a small spiral of bright copper-foil is dropped into the neck. A piece of folded filter paper is then placed in the funnel and the fluid poured into the filter. The stop-cock is opened very slightly, so that about 90 c.c. escapes in a minute. The liquid is collected in a large beaker, which is kept warm by a small flame underneath it, and is re-transferred to the funnel. The fluid should pass over the foil about six times. The entire process can be completed in an hour. The foil is then cleaned as above and dried, and the mercury detected by sublimation. If preferred, the mercury can be collected by electrolysis, using as a negative electrode a small piece of gold-leaf. The amount of current should be about half an ampère. The mercury will all be deposited in about two hours. The fragment of gold-leaf is cleansed and dried in the same manner as given above for the copper-foil, and then heated in a glass tube. After the use of mercury traces of the metal can be found in the urine for a considerable period of time, occasionally for a year.

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[1] Transactions of the College of Physicians of Phila., vol. xxv, 1903, p. 81.

**Methylene Blue.**—The urine is usually colored green, but a blue color can occasionally be produced by boiling the urine with acetic acid. The coloring matter is insoluble in ether, but can be extracted by shaking with chloroform and amyl alcohol. The urine shows an absorption band between *B* and *C*, and another between *C* and *D*.

**Morphine.**—The tests for morphine are preferably applied to the isolated alkaloid.

1. A brilliant red color is given with a mixture of morphine with concentrated sulphuric acid, containing 2 to 3 per cent. of ammonia molybdate (Fröhde's reagent).

2. A dilute morphine solution dropped into a mixture of 1 c.c. of fuming hydrochloric acid, containing a drop of concentrated sulphuric acid gives a purplish red color on evaporation in a porcelain dish at a temperature of about 100 to 120° C. If strong hydrochloric acid is added to the residue, the solution neutralized with sodium carbonate and a trace of alcoholic solution of iodine added, the fluid assumes a brilliant yellow green. The color is soluble in ether with the production of a purplish tint.

**Naphthalin** is excreted as an $\alpha$ and $\beta$-naphthol glycuronic acid in the urine.

*Tests.*—1. The urine darkens on exposure to the air.

2. A few drops of the urine are layered over 1 c.c. of concentrated sulphuric acid, when a brilliant dark green yellow will be produced at the point of contact, if $\alpha$-naphthol glycuronic acid be present.

3. Urine containing $\beta$-naphthol gives a bluish fluorescence when mixed with a few drops of ammonia or sodium nitrate.

4. Three to four drops of chloride of lime solution and the same amount of hydrochloric acid are mixed with 5 c.c. of the urine, which then assumes a lemon yellow color. If the color be extracted with ether and the extract poured over 1 per cent. watery resorcin solution and shaken with a couple of drops of ammonia, the resorcin is colored bluish green, which is changed on the addition of nitric acid to a cherry red.

**Naphthol** may appear in the urine as an ethereal sulphate when used in large quantities externally.

To test for this substance, 500 c.c. of urine should be acidulated with hydrochloric acid and distilled in a current of steam, the distillate extracted with ether and the extract evaporated to dryness. The residue is dissolved in alcohol mixed with a little animal charcoal and gently warmed, filtered, and the filtrate evaporated. The residue gives a brilliant greenish blue color when warmed with a crystal of chloral hydrate.

**Nitrobenzol** when excreted in the urine may be recognized by its odor of bitter almonds, or may be obtained as anilin by distilling the urine in a current of steam and dissolving the oily mixture, which passes over in alcohol. This alcoholic solution is then reduced with zinc dust and dilute sulphuric acid, which produces anilin. The fluid is rendered alkaline and extracted with ether. The ethereal residue gives a violet color with chloride of lime.

**Phenacetin** is excreted in the urine partly as phenetidin, partly as acetyl-para-amido-phenol, and as a glycuronic acid compound.

1. The indophenol reaction is given, see Acetanilid.

2. Two drops of hydrochloric acid and two drops of 1 per cent. sodium nitrite solution are added to the urine, and then some alkaline watery $\alpha$-naphthol solution and some sodium hydrate; if phenacetin be present, a reddish color will be produced which changes to violet on the addition of hydrochloric acid. It may be necessary to heat the urine for from two to three minutes with one-fourth volume of concentrated hydrochloric acid to split the ethereal sulphate; then proceed as above.

3. Urine containing a large quantity of phenacetin has a marked yellow color, and on the addition of oxidizing agents, such as ferric chloride, assumes a bluish red or a dark green color.

4. Urine containing phenacetin reduces Fehling's on prolonged boiling and is lævorotatory, but does not ferment.

**Phenol** is excreted as a phenolsulphuric or glycuronic acid, and partly as hydrochinon sulphuric acid. The color of the urine is greenish blue, which becomes much darker on exposure to the air. Such urines often contain albumin, casts, and blood. The preformed sulphates are diminished, so that such urine may give only a slight cloudiness on the addition of acetic acid and barium chloride. Tests for phenol are given on page 442.

**Picric acid** may be recognized in the urine by its color or by allowing a skein of wool to remain for some time in the fluid, when it will be stained a brilliant yellow. On warming the urine with potassium cyanide a red color is produced.

**Piperazin** is precipitated by picric acid and by a solution of bismuth iodide in potassium iodide. The bismuth compound can be obtained as a fine red powder, if the phosphates are first separated from the urine by the addition of potassium hydrate and filtering. After acidulating the filtrate with a few drops of hydrochloric acid some of the iodide of bismuth and potassium iodide mixture is added. The solution should be warmed, filtered, and allowed to cool. Rubbing the walls of the vessel with a glass rod will accelerate the formation of the bismuth compound.

**Purgatin** (*Anthrapurpurin-diacetate*).—The urine is of a Burgundy red color, and a white strip of linen soaked in the fluid and dried is stained yellow.

**Purgen** (*Phenolphthalein*).—Purgen can be recognized in the urine by the bright pink color which is produced by adding strong alkali. It also gives a diazo-reaction.

**Pyramidon** gives rise to a bright reddish purple color in the urine, suggesting hæmatoporphyrin, and there may be a sediment of red needles deposited while standing. The red color can be extracted by acetic ether, and has been identified by Jaffé[1] as rhubazonic acid. Glycuronic acid is also increased in amount. Other tests are:

*a.* The coloring matter may be extracted by chloroform.

*b.* A dark brown color changing to amethyst, which is produced by the addition of ferric chloride to the urine, and a violet ring which can be obtained by layering over the urine a dilute alcoholic solution of iodine.

**Quinine.**—*Qualitative Tests:* The alkaloid is excreted unchanged in the urine, and can be isolated by shaking out the fluid with ether after it has been rendered slightly alkaline. The residue, after evaporation of the ether, is dissolved in water and shows a marked fluorescence. If some of the solution is warmed with dilute sulphuric acid and a small amount of tincture of iodine is added, greenish plates with a metallic lustre separate from the fluid (Herapathite).

*Quantitative tests* for quinine[2] in the urine may be made as follows:

Two hundred c.c. of the urine to be tested are placed in a beaker, acidulated with a few drops of sulphuric acid, and treated with about 10 c.c. of dry picric acid. The mixture is allowed to stand for a day and then filtered through filter paper until the filtrate comes through perfectly clear. No cloud should be produced in the filtrate on the addition of a saturated solution of picric acid. In case the filtrate remains cloudy after repeated filtering it may be necessary to add a small amount of white of egg. The precipitate on the filter is collected in a flask, mixed with 50 c.c. of a 3 per cent. potassium hydrate solution, and digested for half an hour on a water bath. After the mixture has cooled, 60 c.c. of chloroform are added and the flask is thoroughly shaken at intervals of at least two hours. Twenty-four hours later the chloroform is separated from the watery fluid in a separatory funnel and evaporated in a tarred flask. The watery solution which remains is shaken out for a second and third time with chloroform, and the extracts added to the original in the weighing flask. The chloroform is then evaporated and the flask dried at about 120° C., and weighed after cooling. The method is roughly quantitative, the error being less than 2 per cent.

**Resorcin** is excreted partly as such in the urine, partly as an ethereal sulphate. It gives tests for naphthalin. Other tests must be applied on the isolated substance.

The urine is evaporated to one-fourth of its bulk; the residue boiled with sulphuric acid and the fluid extracted with ether. The ethereal solution is evaporated, boiled with barium carbonate, filtered, treated with animal charcoal and evaporated. The residue is dissolved in water, and gives a violet color on the addition of ferric chloride and a precipitate of tribromresorcin with bromine water.

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[1] Internat. Beit. z. inner. Med., Bd. ii, p. 1; see also *Apert:* Arch. gén. d. méd., 1904, p. 1665.

[2] *Kleine:* Zeit f. Hygiene, Bd. xxxviii, 1901, p. 458.

**Rhubarb** colors the urine a deep yellow or a greenish yellow, from the chrysophanic acid contained in this drug. Senna gives the same color.

*Tests :* 1. The yellowish colored urine becomes purple red on the addition of alkali and the precipitate which is formed has a reddish color. The color of the precipitate is unaltered by the addition of acids, which distinguishes the reaction from the Heller test for blood.

2. Urine colored by chrysophanic acid and of an acid reaction is decolorized when shaken with half of its bulk of ether by the solution of the acid in the ether, and the ethereal solution assumes a red color on the addition of sodium hydrate. This reaction differentiates between urine containing santonin, the color of which is not extracted by ether. Another difference lies in the fact that the red urine containing rhubarb and senna when treated with dilute acid and zinc dust is decolorized, while santonin urine is not. Chrysophanic acid is precipitated by baryta water and milk of lime, while santonin urine retains its color.

3. Acidulate the urine with hydrochloric acid and shake out with chloroform, when, if chrysophanic acid be present, a violet ring will be produced on the addition of potassium hydroxide.

**Saccharin** appears unchanged in the urine. The fluid to be tested for saccharin is acidulated with phosphoric acid and extracted with ether. The ethereal residue has an extremely sweet taste, and if it is heated at 250° C. for half an hour with a small quantity of concentrated sodium hydrate solution, and after acidulation extracted with ether, the residue, after evaporation of the ether, gives the reaction of salicylic acid.

**Salacetol, Salipyrin, Salophen, Salosantal,** and **Salol,** all give the reactions of salicylic acid.

**Salicylates** appear in the urine as salicyluric acid, as ethereal sulphates or as glycuronates, and partially in unaltered form. The most important test is the bluish violet color, which appears on the addition of ferric chloride as in Uffelmann's test for lactic acid. If only small quantities of salicylic acid be present, the urine should be acidulated with sulphuric acid and shaken out with ether, the ether allowed to evaporate spontaneously and the residue tested with very dilute ferric chloride solution, or the ether may be shaken with dilute sodium hydrate and the latter mixed with a dilute solution of ferric alum.

**Sandalwood oil** is excreted in the urine as a glycuronic acid compound and may be recognized by the same test as for copaiba, but distinguished from the latter by the fact that the addition of mineral acids does not give rise to a red color.

**Santonin** causes the urine to assume a yellowish color, which is due to xanthopsin, which changes to a red on addition of alkali and is not extracted by ether. (See Rhubarb.)

**Sulphonal, Trional,** and **Tetronal** in large doses are likely to incite hæmatoporphyrinuria. The identification of the pigment depends upon spectroscopic tests and is given on pages 22 and 572.

**Tannin** and **Tannigen** appear in the urine as gallic acid, which gives a greenish-black color with ferric chloride. The alkaline urine turns black from the absorption of oxygen.

**Terpin hydrate** may be recognized in the urine after distilling 500 c.c., extracting the residue with 5 c.c. of alcohol, and heating the alcoholic extract so as to cause the vapor to pass over a crystal of subchloride of antimony. The latter assumes a red color if terpin hydrate be present.

**Thymol** appears in the urine as thymol-sulphuric acid, thymol-glycuronic acid, and as a green coloring matter. Urine containing large quantities deposits crystals of dichlorthymol-glycuronic acid when mixed with a third of its volume of concentrated sulphuric acid and sodium hypochlorite.

**Urethan** when it appears in the urine reduces Fehling's. If 500 c.c. of the urine is shaken out with ether, the ether washed with water and the residue obtained on evaporation of the ether dissolved in water, the solution when rendered alkaline with potassium hydrate will give a precipitate of yellow mercuric oxide, which changes on agitation to a white mercuric carbamate.

**Urotropin** gives an orange-red colored precipitate of dibromurotropin when the urine containing it is mixed with bromine water.

## IV. MICROSCOPIC EXAMINATION OF URINE

### THE SEDIMENTS IN THE URINE

The sedimentation of the urine for microscopical examination may take place spontaneously in a conical glass, or it may be hastened by the use of a centrifuge. The rapid method is far more preferable, as there is no chance for any alterations to occur in the deposits. Especially is this true in strongly alkaline urine, in which hyaline casts may be dissolved in a very short time. The casts and other morphological elements which may be present are not injured by centrifugalization.

The deposit is to be obtained from the conical tip of the glass by means of a pipette with a rather finely-drawn-out tip. The finger is placed over the upper end of the pipette and the tip is lowered until it is in contact with the deposit; the pressure on the upper end of the pipette is then removed and the deposit allowed to flow rapidly into the lumen of the tube. The finger is then tightly pressed over the upper end, and by gradually rotating the pipette small amounts of the urine are allowed to escape on the slide for examination. Hollow ground slides are not so suitable as those of plain glass of extra width. The size 2 by 3 inches is the best. The large slide is preferable, because the urine is less liable to run over the edge and soil the surface of the microscope stage.

A cover glass has the advantage that it renders the soiling of the front lens of the objective less likely, but it is prone to alter the morphology of the more delicate elements by pressure unless it is carefully adjusted. A mechanical stage for the routine searching of a preparation is very convenient.

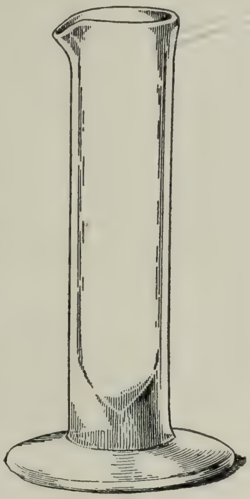

Fig. 154.—A Convenient Form of Cylinder for Sedimenting Urine.

The lens to be used is a comparatively low power one with a focal length of 8 to 16 millimeters. Of the lenses on the English system, a half inch is the most suitable. A moderately high eyepiece is advisable. With this combination the large field allows the rapid searching of a large surface, and yet, if the light is properly adjusted, the casts, crystals, and epithelium are much more easily seen than with a higher power. The beginner always makes the mistake of working with a 4 or 6 millimeter dry lens, so that, besides the discomfort of the small field and the tiring of the eyes which results from the rapid movement of the objects because of the high magnifying power, there is the need of great care in handling the lens lest it become injured by contact with the urine or reagents. If crystals are being tested with acids a very large cover glass should always be used and a low power objective, if possible, in order not to get the mount too close to the corrosive fumes.

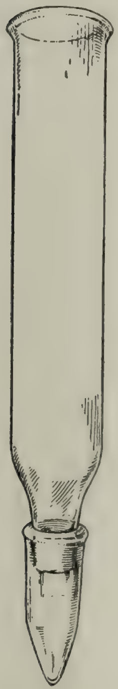

Fig. 155. — Improved Centrifuge Tube with Removable Tip.

The acid or other reagent to be used is placed at one side of the cover glass by means of a pipette and drawn under the cover by abstracting urine from the opposite side by a narrow strip of blotting paper.

The regulation of the light is a most important matter. As most of the modern stands are fitted with an Abbe condenser, the question is almost always one of diminution in the amount of light to the smallest amount possible by the use of the smallest diaphragm, or by reducing the iris to the smallest aperture. It is often of advantage to use a low artificial light rather than brilliant daylight. If the Abbe can be removed entirely and the light then reduced by a diaphragm, the conditions will be most favorable to the beginner in his search for hyaline casts, though this procedure is not necessary after some experience.

The preservation of urinary deposits as type specimens is a difficult matter. The crystals are best mounted in glycerine jelly.[1] Some of this is melted and poured over the centrifuged deposit. A drop of the warm mixture is then placed on a slide, covered with a cover glass and allowed to cool. The preparations must be ringed with asphalt or shellac. Casts and epithelium may be preserved by sedimenting the urine and washing the sediment in several changes of physiological salt solution until the albumin is removed, and then hardening them in a 5 per cent. formaldehyde solution. They may be kept fairly well in this way in solution, and they may be mounted in glycerine jelly, but the results are unsatisfactory except for the coarser varieties of casts, such as the waxy and epithelial varieties.

### ORGANIZED DEPOSITS

**The red blood corpuscles** in fresh acid urine preserve their shape and color very well for a short time. Later the hæmoglobin is likely to be washed out by the solvent action of the fluid. In alkaline urine or in very dilute urine the color quickly fades and the cell swells up and is visible only as a faint ring. In concentrated urine the cell assumes a crenated form such as is seen in a fresh blood preparation after concentration of the serum has taken place from evaporation.

**Leucocytes** retain their structure very well in acid urine, and by the addition of a little acetic acid the nucleus can be rendered quite distinct. In alkaline urines the leucocytes lose their shape quickly and undergo solution in the ammoniacal fluid, forming a stringy mass of nucleo-albumin and cell remnants which is often called mucus, and which becomes much more stringy if a little strong NaOH be added. The pus in urine will blue freshly prepared guaiacum tincture if the latter is layered over the purulent fluid.

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[1] See Appendix, page 747.

**Mucus** is present in very small quantities in the urine in the nubecular cloud and in small threads, which are often branched and taper at the ends into smaller threads. These threads are not nearly so refractile as the faint hyaline casts and are easily distinguished from them by their tapering ends.

**Epithelium** is present in the sediment of every urine, rather more abundantly in the urine from females owing to the large proportion of vaginal epithelium. The epithelium from the female urethra, the vagina, and the male urethra is often large in size and oval in shape, with a small nucleus compared to the size of the cell. The vaginal is usually larger, though an occasional large cell may

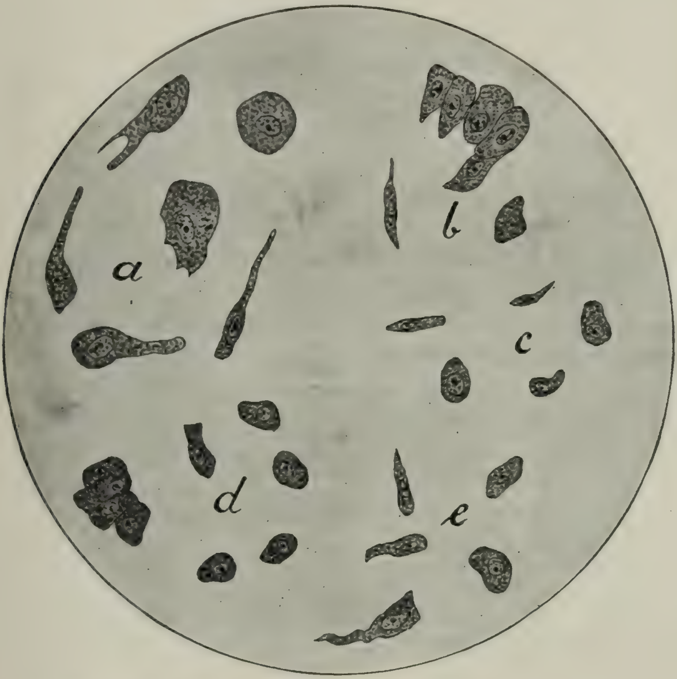

Fig. 156.—Epithelium from Genito-urinary Tract.
*a*, Bladder; *b*, near neck of bladder; *c*, urethra; *d*, kidney epithelium; *e*, ureter.

come from other regions. The cells from the superficial layers of the bladder may also be rather large, but the nuclei are usually slightly larger than that of the vaginal epithelium. Small round cells may come from the whole of the urinary tract, but when they are dark in color and have large nuclei and are very granular they are generally assumed to be derived from the kidney tubules. This supposition can only be proven when this form of epithelium is passed in the urine in the form of cylinders or casts of the urinary tubules. The isolated cells can not be certainly identified. The fact that the kidney is the seat of an active nephritis, with much albumin in the urine, will, of course, render the probability that an isolated cell is derived from the kidney epithelium somewhat greater.

Epithelium of irregular shape and cells with long tail-like projections may be derived from any portion of the urinary tract, but usually from the deeper layers.

It is impossible to determine exactly the site of production of a given epithelial cell. For a long time it was considered that certain of the tailed cells were derived from the pelvis of the kidney, but this view has been abandoned. The only fact of diagnostic value to be derived from an examination of the morphology of urinary epithelium is that there is present in the urine a greater or a less amount, and that this is to a certain extent an index to the amount of inflammation and desquamation going on.

The epithelium in the urine may undergo degeneration before it is set free in the fluid. The commonest form of degeneration seen is fatty change, especially in the small granular cells from the tubules of the kidney. The fat granules can be recognized more easily if the deposit be treated with 1 per cent. osmic acid or with an alcoholic solution of Sudan III or scharlach R, all of which color the fat. A convenient mixture for the purpose is one obtained by mixing two parts of a saturated solution of Sudan III in 70 per cent. with one part of 10 per cent. formalin, and filtering.

**Fragments of tumors** may be passed in the urine, and if imbedded and sectioned may cast some light upon the nature of a growth in the urinary tract; but, as a rule, such fragments are too necrotic to permit of an accurate opinion from the examination of a section. The same condition holds true if the specimen is examined fresh.

**Spermatozoa** are occasionally found in the urine of the male after coitus or a nocturnal emission, and in spermatorrhœa. They are also found after an epileptic attack. In the female they are present in the vagina and occasionally in the urine for some days after coitus.

## CASTS

When the substance of the kidney is altered by circulatory, toxic, or inflammatory disturbances, the urine contains cylindrical bodies which originate in the tubules. Besides these true casts we may find cylinders in the urine of a slightly different morphology which do not necessarily denote any of the above conditions. The first are called casts, and the second cylindroids and mucous threads. It will be more convenient to begin with the latter.

**Mucous threads** are bodies which appear in the normal urine in the form of long, tapering, transparent shreds which are longer and usually thinner than casts of the hyaline variety, for which they are most likely to be mistaken. They frequently taper out at both ends in the form of fine threads, or they may split into several fine divisions and these separate tails fade away imperceptibly. The mucous threads are very often covered with fine

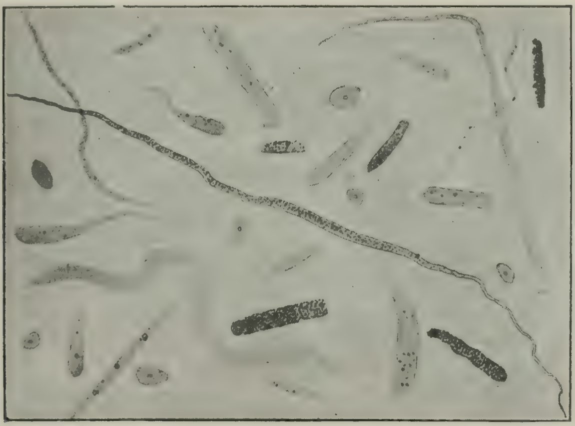

FIG. 157.—HYALINE AND GRANULAR CASTS, MUCOUS THREADS AND CYLINDROIDS. Scattered through the Field are a few Epithelial Cells from Bladder.

granules deposited on them as the urine cools and the urates begin to separate out. They should never be mistaken for casts.

**Cylindroids** are rather larger and more refractile cylinders than the mucous threads, and at times can not be distinguished from true hyaline casts except by the fact that the cylindroid tapers out gradually at one or both ends into a long tail, while the casts possess a rounded or short pointed end. The cylindroids are also, as a rule, a little broader than hyaline casts. They may also collect granular deposits and occasionally have leucocytes attached to them. An absolute and dogmatic distinction between a cylindroid and a given hyaline cast is at times quite impossible. They both appear in the urine under much the same conditions; that is, in cases of chronic nephritis without much albumin and with only a few hyaline casts, and in the urine of old people. Occasionally a true hyaline cast may be seen with a short tail instead of a rounded end, and in such a dilemma it is always necessary to search the urine further until definite forms are discovered which will enable one to say that casts are or are not present.

**Hyaline casts** are the most difficult to find, but at the same time they are the most important form of casts from a point of view of diagnosis. They are often present in very small numbers, and disappear from the urine so rapidly when the fluid undergoes any change that they are often overlooked. The urine, therefore, in an important case, should always be centrifuged as soon as possible after passing, and the deposit examined with great care as to the proper regulation of the amount of light used. It is better to have too little than too much; the first condition can only strain one's eyes, the second will insure many a faulty diagnosis.

The importance of hyaline casts lies in the fact that they are often the only evidence of an advanced nephritis, the discovery of which would deter the surgeon from operating, or decide the question of life insurance, or of suitable climatic or medical treatment.

Hyaline casts are narrow, cylindrical bodies of about the same proportions in regard to length and breadth as the human finger. They are usually rounded at each end or cut off obliquely; but as a rule there is no sharp point and especially no prolonged tail. (See Fig. 157.) Hyaline casts are often covered with granular matter and at times with leucocytes, but a careful examination with a high power will show that the deposits are on the cast and do not form a part of its substance, as is the case with the granules in the true granular casts. This distinction should always be made, as the prognostic value of granular casts is far graver than that of the hyaline casts. Hyaline casts rarely contain in a part of their length cast material of a different sort, but occasionally a cast is seen which is hyaline at one end and granular or composed of blood at the other. (See Fig. 157.)

**Granular casts** are distinguished from the hyaline variety by their deeper color and greater refracting power, as well as by the fine or coarse granules which are contained in their substance. (See Figs. 157 and 158.) The lateral borders are also rather inclined to be irregular and to show a slight waviness. They frequently are granular at one end and quite transparent at the other, and may contain red or white blood cells or epithelium in small amount or may be fatty.

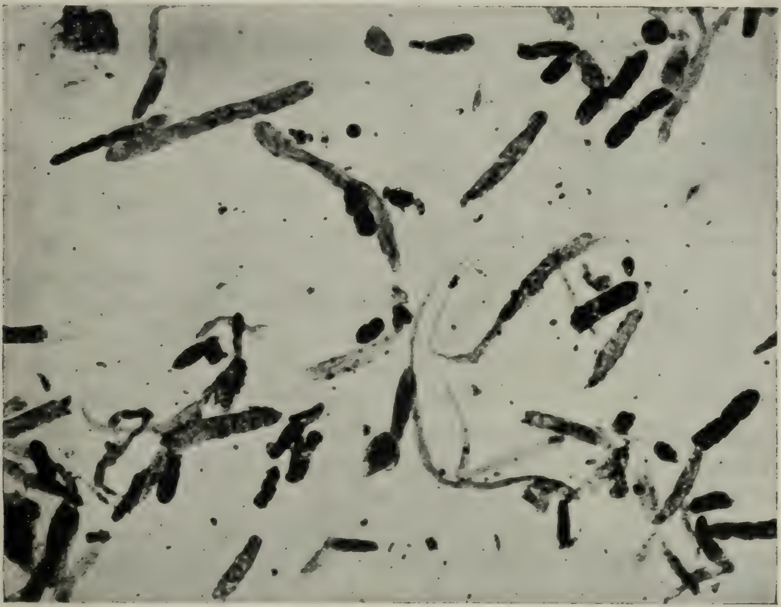

Fig. 158.—Finely Granular Casts from Case of Subacute Diffuse Nephritis. (200 diameters.)

**Epithelial casts** are, as the name indicates, composed of tubular epithelium with a more or less transparent matrix.

**Blood, leucocytes,** and **pus casts** are sufficiently described by the name.

**Waxy casts** are very large, highly refractile casts which are not very frequent in the urine, and which occasionally give the reactions for amyloid with iodine or the aniline dyes. The ends show a well-marked fracture, and the casts have a characteristic well-defined and wavy outline which is sometimes due to a spiral twist in the entire cast. They are very resistant to the action of acid. Transitions from wax to epithelium or granular masses in the same cast are common. (See Fig. 160.)

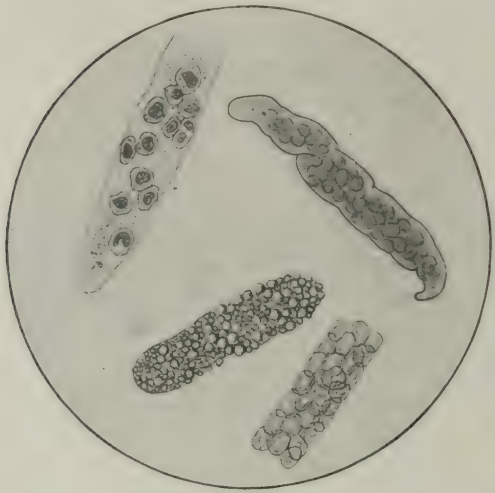

FIG. 159.—EPITHELIAL, COARSELY GRANULAR AND FATTY CASTS.

DIAGNOSTIC VALUE OF THE DIFFERENT VARIETIES OF CASTS

**Hyaline casts** are not always indicative of an active or progressive nephritis. They may be found in about 50 per cent. of cases of general anæsthesia if care is taken to centrifugalize the urine and make a thorough search. Evidently the lesion is a temporary irritation or possibly a congestion of the kidneys, as is shown by the fact that the casts, and the trace of albumin which often accompanies them, disappear from the urine in twenty-four to forty-eight hours.

Casts of a hyaline nature are found in the urine of many persons over fifty years of age, but with no other evidence of a renal lesion, and very rarely without albumin being present.[1] As a rule this condition is not regarded as indicating a chronic diffuse nephritis, but only that a small area of one or both kidneys may have a few slight changes, which will account for the presence of these casts, and the trace of albumin which may or may not accompany the latter. Any one who has sectioned and examined microscopically many kidneys from elderly persons will agree that a perfectly normal kidney in such people is almost never seen, but that frequently small areas will show a little connective tissue and a few atrophic glomeruli.

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[1] See, in this connection, *Osler:* "On the Advantages of a Trace of Albumin and a Few Tube Casts in the Urine of Certain Men Above Fifty Years of Age," New York Medical Journal, vol. lxxiv, 1901, p. 949.

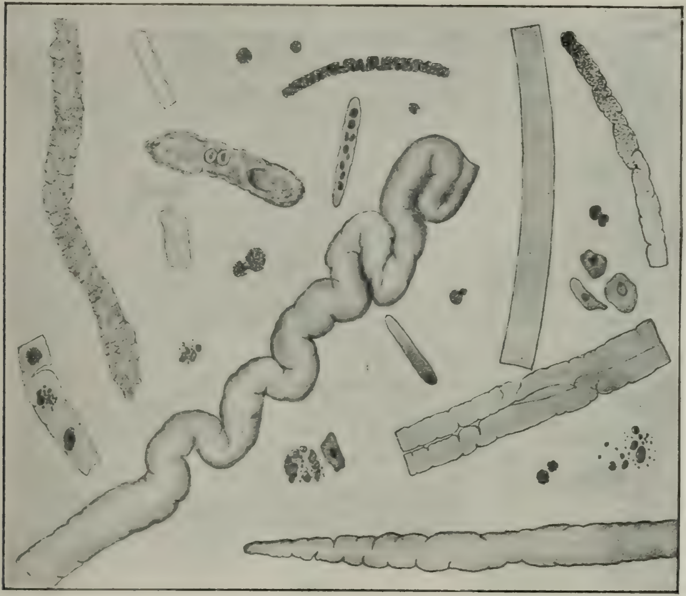

FIG. 160.—TYPES OF CASTS WITH A WAXY MATRIX FROM A CASE OF SUBACUTE PARENCHYMATOUS NEPHRITIS. Some of the casts are quite transparent, others are granular at one end and clear at the other. Some are composed partly of granular matter and partly of waxy material. The casts vary greatly in size but are all drawn to the same scale. One very small cast in the center is of the hyaline variety.

Hyaline casts are also found in all cases of well-marked jaundice; and here they also seem to be the evidence of a transient lesion of the epithelium, for they, as well as the traces of albumin and nucleo-albumin usually present, soon disappear from the urine when the excretion of bile through the kidney ceases.

Hyaline casts are also seen in febrile diseases, in chronic congestion of the kidneys, in severe anæmias, and in diabetes. In all these conditions the renal changes found on autopsy are slight, and the casts or the trace of albumin with them seem to be due to the altered nutrition or degeneration of the tubular or glomerular epithelium. This does not mean that a true and active nephritis may not be seen under the above conditions. Such is often the case, but what is meant is that a very few hyaline casts may be seen without the diagnosis of nephritis being necessary.

In the great majority of cases, however, the presence of hyaline casts and albumin means the existence of a chronic nephritis. In general the greater the number of casts the more severe the disease; but there are many exceptions, and a patient may pass urine nearly normal in quality when the kidneys are in an advanced state of nephritis and uræmia is threatened.

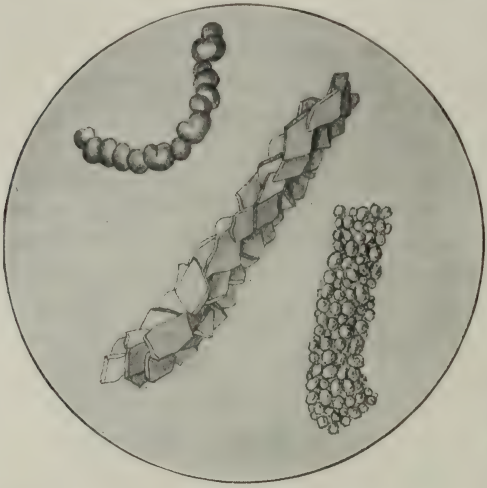

Fig. 161.—Pseudo-casts of Ammonium Urate and Uric Acid.

The casts may appear before the albumin, and may remain after the albumin has disappeared in cases of mild chronic nephritis which are amenable to treatment.

**Granular casts** invariably indicate a nephritis of severe grade, especially when the granules are coarse.

**Waxy casts** are not necessarily the accompaniment of a waxy kidney; in fact, they are rather uncommon in this condition, but are seen most frequently in the large white kidneys which often show a moderate amount of waxy degeneration, accompanied by advanced changes in the glomeruli and tubular epithelium. They appear also in acute, rapidly fatal cases of nephritis.

**Epithelial casts** indicate a severe destructive lesion of the kidney epithelium, and are only rarely seen except in most advanced nephritis.

**Blood casts** are chiefly seen in acute glomerular nephritis with hæmorrhage from the vessels of the tuft, and indicate a serious lesion of the kidney.

**Pus casts** are met with most frequently in acute pyelo-nephritis, and are of grave diagnostic import.

## CRYSTALLINE AND AMORPHOUS DEPOSITS IN THE URINE

The unorganized deposits in the urine can be conveniently divided for clinical purposes into those which appear chiefly in acid urine and those which are found in alkaline urine. It is important to remember that the addition of preservatives to the urine may alter the form of the crystals or give rise to new varieties. For example, it has been shown[1] that the addition of formalin to urine may give rise to disk-like bodies about twice the size of a red corpuscle, with radial striations, which makes them resemble leucin. These crystals are a compound of formalin and urea.

### I. SEDIMENTS IN ACID URINE

1. The quadriurates of sodium and potassium form the chief portion of the yellow or brick-red deposits which occur in any concentrated acid urine. The color of the yellow deposit is due to urochrom and urobilin, that of the red to uroerythrin. The deposit frequently contains traces of hæmatoporphyrin. Microscopically the deposit is formed of a finely granular sediment, which can be decomposed into uric acid and a biurate by the addition of a little water. The deposit is soluble on warming the urine slightly, and is easily soluble in acetic acid with the separation of uric acid crystals. The urates all give the murexide reaction.

2. Uric acid separates from acid urine on the breaking up of the quadriurates into the biurates and uric acid; the latter is then deposited in the form of rhombic or whetstone-shaped plates or six-sided prisms with rounded angles. The smaller forms of the rhombic plates are at times colorless, but almost all the other forms are deeply colored with the urinary pigments. Dumb-bell forms are occasionally seen, and large rosettes formed by the combination of the prismatic crystals are very common. Uric acid is not soluble in acetic acid nor on heating, but can be dissolved by potassium hydrate or by a 10 per cent. solution of piperazin, and

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[1] *Smith :* Practitioner, 1903, p. 160.

the uric acid again separated from the solution by the addition of a little HCl. The murexide reaction is given.

3. Calcium oxalate appears most frequently in acid urine, but may often be found after a urine has undergone alkaline fermentation, owing to its insolubility in alkali. It is characterized by the small, highly refractile octahedra with a cross connecting the corners. Occasionally one sees twin crystals with a half-rotation of one of the components, causing one octahedron to have small projecting points from the side, these points being the angles of the other crystal. Pyramidal prisms or oval or dumb-bell forms are not so frequent as the octahedra. Calcium oxalate is insoluble in acetic acid, but soluble in HCl. (See Fig. 163.)

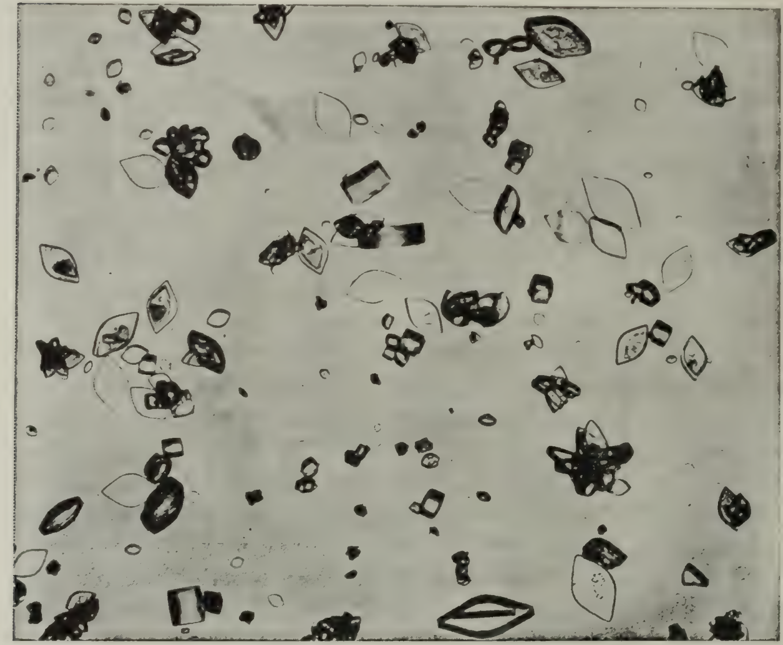

Fig. 162.—Crystals of Uric Acid.

4. Cystin is a product of proteid metabolism which appears in the urine in the form of highly refractile six-sided tables. (See Fig. 165, p. 605, and Fig. 136, p. 460.) They are soluble in HCl, in alkalies, ammonia (point of difference from uric acid), and are insoluble in acetic acid. They are very rarely found in the

urine, and attention is usually called to the condition by the finding of a cystin calculus in the genito-urinary tract.

5. Leucin appears in the urine in the form of yellow, oily-looking spheres with a radial striation. It is not soluble in hydrochloric acid, which separates it from ammonium urate, nor in ether. The latter reaction, and the fact that it does not stain with Sudan III or scharlach R, differentiate leucin from globules of fat.

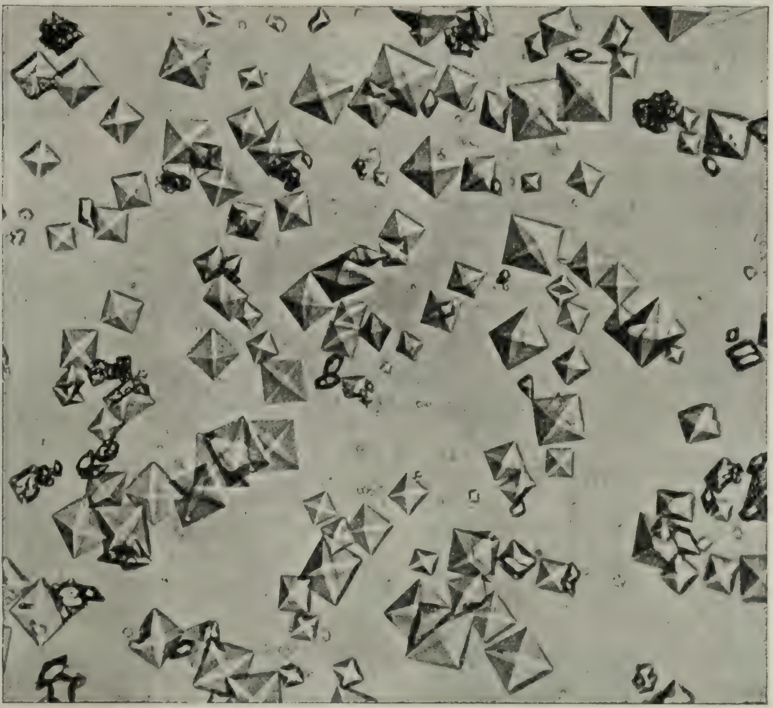

Fig. 163.—Crystals of Calcium Oxalate.

Tyrosin forms bundles of fine needles which are soluble in ammonia and HCl, but not in acetic acid. It gives Millon's reaction.

If both leucin and tyrosin are present in large quantities in the urine they may form a deposit, but usually it is necessary to evaporate some urine to about one-tenth of its original volume and then add some alcohol to the solution and examine under the microscope for the formation of the characteristic crystals.

Leucin and tyrosin should always be tested chemically, and not identified solely by their morphology. (See Fig. 135, p. 457.)

6. Bilirubin or hæmatoidin appears in the urine in the form of brilliant yellow rhomboids or yellow needles. It may also form a yellow granular deposit. The rhomboids sometimes have small, curved needles projecting from the angles. It is soluble in chloroform and potassium hydrate and gives Gmelin's reaction.

7. Hippuric acid has been noted several times in acid urines as transparent colorless prisms. (See Fig. 134, p. 453.) The crystals are soluble in alcohol and also ether, which distinguishes them from uric acid.

### II. SEDIMENTS IN NEUTRAL AND AMPHOTERIC URINE

In addition to the sediments described above, neutral calcium phosphate may be occasionally found. The crystalline form is that of slender pyramids united by their apices to form rosettes or cross-shaped figures. (See Fig. 165.) They are soluble in acetic acid, and form balls of calcium carbonate when treated with 20 per cent. ammonium carbonate. Ammonium-magnesium phosphate may rarely be found in neutral urine.

### III. SEDIMENTS IN ALKALINE URINE

1. Amorphous deposits of tricalcium and trimagnesium phosphates are often very abundant in alkaline urine. They are white, bulky precipitates which are soluble in acetic acid without evolution of carbonic acid gas.

2. Calcium carbonate, which may also form an amorphous precipitate or groups of large spheroidal masses, gives off $CO_2$ when treated with acetic acid.

3. Ammonium urate is quite frequently present in alkaline urine in the form of dark brown or black spheres, either single or united, and frequently covered with short, curved projec-

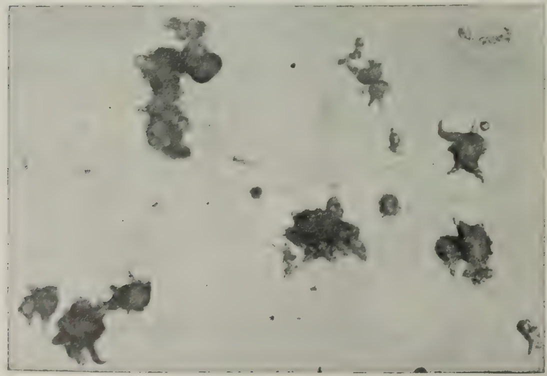

FIG. 164.—CRYSTALS OF AMMONIUM URATE.

tions forming the so-called thorn-apple crystals. They dissolve in hydrochloric acid with the separation of uric acid and give the murexide test. They dissolve in strong alkalies with the evolution of ammonia.

4. Triple phosphate or ammonio-magnesium phosphate crystals are the most frequent crystalline forms in alkaline urine. They appear either as the coffin-lid forms, or as long, feathery crystals, of which four are usually united by their bases, forming a mass like a rough letter H. The coffin-lid forms are exceedingly characteristic, but can be still further identified by their easy solubility in acetic acid.

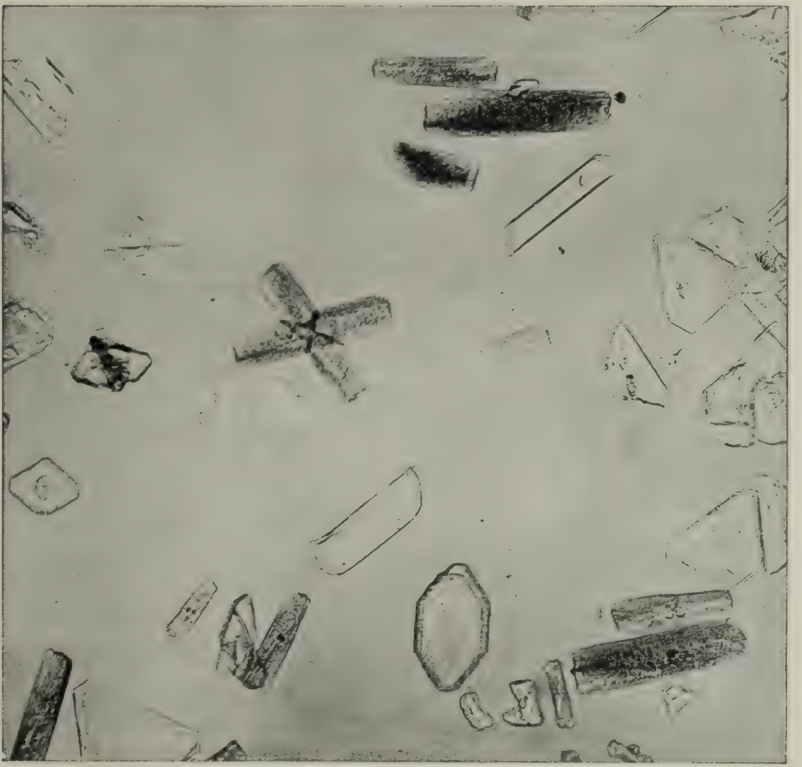

Fig. 165.—The Cross-shaped Crystal in the Center is Calcium Phosphate. The Hexagonal Crystal in the Lower Portion of the Figure is Cystin. The Irregular Transparent Crystals to the Right are Ammonium-magnesium Phosphate.

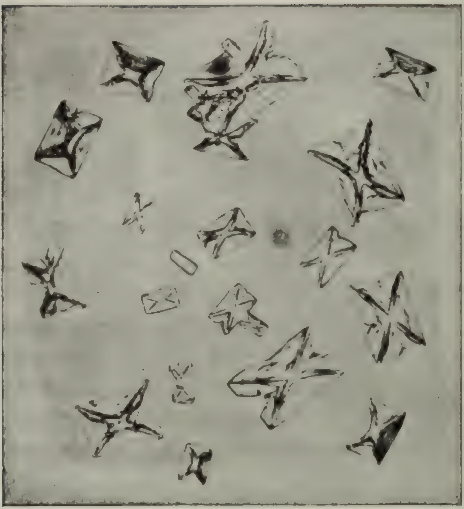

Fig. 167.—Crystals of Ammonium-magnesium Phosphate.

The summary which is given below may also be used in testing unknown deposits.

1. Colorless crystals may consist of calcium phosphate, calcium sulphate, ammonium-magnesium phosphate, calcium oxalate, cystin, xanthin, tyrosin, or hippuric acid.

Calcium sulphate and tyrosin form fine needles; cystin, six-sided or rhombic plates, or rarely, thick needles; calcium phosphate,

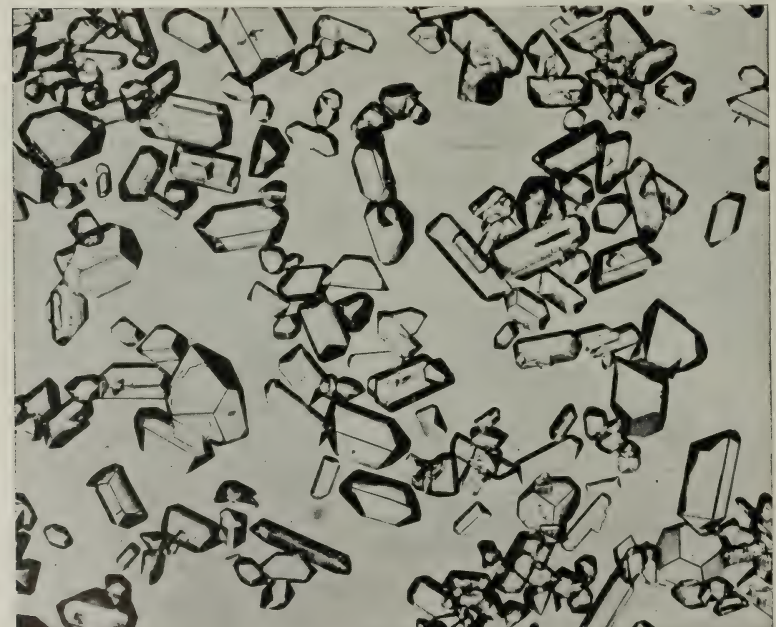

Fig. 166.—Crystals of Ammonium-magnesium Phosphate.

rhombic prisms; xanthin, six-sided plates or small oval flat crystals; calcium oxalate, octahedral or long prismatic bodies. Ammonium-magnesium phosphate forms large six-sided prisms with oblique end facets. Short prisms of the triple phosphates may resemble the octahedra of calcium oxalate, but these forms are rare.

2. Colorless balls or dumb-bells consist of calcium carbonate, oxalate or sulphate.

3. Yellow, reddish or brown crystals are uric acid, and very rarely phosphates or oxalates colored with bile pigment.

4. Yellow and reddish brown balls or masses with prickle or spur-like projections are either urates or leucin.

5. Calcium and magnesium phosphates, alkaline urates and xanthin form fine granular deposits.

### THE RELATION OF THE SEDIMENTS TO REAGENTS

1. Strong acetic acid dissolves calcium and magnesium phosphates, calcium carbonate and ammonium-magnesium phosphate; but does not dissolve calcium sulphate or oxalate, uric acid, xanthin, or cystin. Uric acid salts are partially dissolved and leave a residue of uric acid crystals, which are better marked after an hour or two than just after making the test.

2. Hydrochloric acid dissolves all crystals except uric acid and calcium sulphate.

3. Ammonia dissolves tyrosin, cystin, and xanthin. Uric acid crystals are partially eroded with the formation of ammonium urate.

4. Water sets free uric acid from the urates of sodium and potassium, and when in great excess dissolves tyrosin, xanthin, uric acid and urates: but this solution is hardly to be noticed in microscopical preparations.

5. A 20 per cent. solution of ammonium carbonate is useful in differentiating between calcium and magnesium phosphates and triple phosphate. The last remains unchanged. Magnesium phosphate crystals are eroded and small triple phosphate crystals are soon formed by the action of the ammonia. Small globules of calcium carbonate are formed on the calcium phosphate crystals.

6. Chloroform dissolves bilirubin and fat.

7. Alcohol dissolves tyrosin, leucin, cystin, and hippuric acid.

## CALCULI IN THE GENITO-URINARY TRACT AND THEIR ANALYSIS

The formation of urinary calculi depends upon the same physical and chemical changes in the urine as give rise to the crystalline and amorphous sediments. The latter are rarely found in freshly passed urine, but form rapidly when the urine cools or changes its reaction. So, too, when bacteria set up an alkaline fermentation in the urine, particles of the phosphates may be deposited upon any foreign substance which may be present in the genito-urinary tract and form a calculus. The process of accretion may stop before the stone becomes too large to pass the ureter or the urethral orifice, or it may continue and give rise to very large masses which partly fill the pelvis of the kidney or bladder. The foreign substances which form the nucleus of a calculus may be a small crystal of uric acid or calcium oxalate, for example; or it may be a flake of fibrin, or mucus, a small blood clot, a few desquamated epithelial cells, or finally some wholly foreign matter, such as the tip of a catheter or a non-absorbable suture. The growth of the stone takes place by the deposition of successive layers upon the original nucleus. In the calculi formed of cystin, xanthin, uric acid, or calcium oxalate, the layers may be of the same nature as the nucleus, but usually the different strata vary considerably in their chemical composition. Calculi also vary considerably in their color, form, and hardness, depending upon their chemical composition.

1. **Uric acid and urate calculi** are among those most frequently met with. They are usually composed of a mixture of these two substances, are yellowish or brownish in color, with a slightly roughened surface and fairly hard. On cross-section they show a distinctly laminated structure. Some of the small calculi are composed of crystalline aggregations of nearly pure uric acid, with very sharp angles. Ammonium urate calculi are small, yellow, and very soft. All of this group give off the odor of hydrocyanic acid when ignited on platinum-foil and leave but little residue.

2. **Calcium oxalate calculi** are very hard and show a crystalline fracture. The smaller ones are smooth-surfaced and white; the larger have a very rough exterior, with sharp projecting angles, and are often stained a dark brown color from blood pigment derived from the hæmorrhages produced by the sharp angles of the stone (mulberry calculi). These calculi are changed into

calcium carbonate and lime on heating. They are soluble in hydrochloric acid.

3. **Phosphatic calculi** are composed of ammonium-magnesium phosphate and the normal phosphates of the alkaline earths, calcium and magnesium. They often contain an admixture of ammonium urate and calcium carbonate, occasionally some calcium oxalate. Pure calcium phosphate and triple phosphate stones are rare. Stones of the phosphate group are often of large size, of a white or yellowish color and of a chalky consistency. The surface is usually rough. They are soluble in hydrochloric acid, and leave an abundant white residue on heating.

4. **Calcium carbonate calculi** are rare. They are small, have a chalky feel and a smooth white surface, and are friable.

5. **Cystin calculi** are of a pale yellow color, have a smooth or mammillated surface, and are soft and friable. The cut surface is translucent and waxy. Flat crystalline plates can often be split off from the broken surface of the calculus.

6. **Xanthin calculi** are very rare. They are light brown in color and moderately hard. When the surface of the stone is rubbed it takes a dull, wax-like polish, and flat plates can be split off from it. They leave but little residue on heating and are dissolved by hydrochloric acid.

7. **Cholesterin calculi** are very rare. They resemble cystin stones in their gross appearance, and are soluble in a mixture of alcohol and ether. When this solution is evaporated rhombic plates of cholesterin are obtained which can be identified by their form and reactions (page 338).

8. **Urostealith calculi** are small masses composed chiefly of fatty acids and fat, the former combined with calcium and magnesium. The analyses of Horbaczewski[1] showed that the calculi which he examined contained 51.5 per cent. of fatty acids, chiefly stearin and palmitin, and 31.5 per cent. of fat.

9. A calculus composed of **indigo** has been described. It weighed forty grams. Indigo can be sublimed by slow heating, and dissolves in chloroform with the production of a blue color.

10. **Mixed calculi** are frequent. They consist of concentric layers of substances of varying chemical composition, most frequently uric acid, calcium oxalate, and the phosphates. Rarely

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[1] Zeit. f. phys. Chem., Bd. xviii, p. 335, 1893.

such stones contain slight admixtures of cystin, tyrosin, xanthin, or even small amounts of indigo red or indigo blue.

### THE QUALITATIVE ANALYSIS OF URINARY CALCULI

A small quantity of the finely pulverized stone is heated on a small porcelain dish or platinum foil. If the powder burns completely or leaves behind only a small quantity of ash, the stone consists of either uric acid, ammonium urate, cystin, xanthin, indigo, or proteid substance, or is the exceedingly rare stone composed of fats, known as urostealith. Urate and xanthin calculi give off the vapor of hydrocyanic acid, which can be recognized by its odor. If the combustion is not complete the stone may possibly contain some uric acid or its salts, besides calcium or magnesium phosphate, ammonium-magnesium phosphate or calcium oxalate. Cystin, xanthin, and urostealith stones contain little or no inorganic matter. The further course of the analysis depends upon the results obtained by heating the stone.

I. The calculus leaves no residue on heating, or only a very slight one.

A fresh portion of the powder is warmed with 30 per cent. hydrochloric acid.

*a*. The powder dissolves completely, or almost completely, in which case the stone consists of cystin or xanthin.

To identify cystin, a small fragment of the powder is dissolved in ammonia, the solution filtered, and allowed to evaporate slowly in a watch-glass. The residue when examined by the microscope will show flat hexagonal crystals.

To test for xanthin, dissolve a small quantity of the powder in nitric acid and dry carefully over a small flame. In case xanthin is present a lemon yellow residue is left, which becomes intensely red when touched with a drop of concentrated sodium hydrate. If water be added to the mixture a yellow solution is produced which on being evaporated again leaves behind a violet red residue. (Difference from uric acid.)

*b*. The powder does not dissolve completely in hydrochloric acid. The mixture is filtered and the residue is washed with water.

1. The residue on the filter is uric acid or organic matter. The former can be identified by the murexid reaction: Evap-

orate at a gentle heat a little of the powder with some nitric acid in a porcelain dish. The residue assumes a beautiful purple-red color when exposed to the fumes of ammonia, the color changing to violet when treated with sodium hydrate solution.

2. The filtrate contains ammonium chloride. To test for ammonia, warm the solution with sodium hydrate, when fumes of ammonia will be given off, and can be identified by their odor and by bluing a moistened strip of red litmus paper.

II. The calculus blackens, but combustion is not complete. Almost all urinary calculi turn black on heating, because of a small quantity of organic substance which is contained in practically all of them. A fresh quantity of finely powdered stone is heated with 30 per cent. hydrochloric acid. The evolution of gas indicates the presence of carbonates.

*a.* The solution is complete, which indicates that uric acid is absent.

*b.* The solution is incomplete.

The residue consists of uric acid, albuminous substances, epithelium, etc. The examination of the residue under the microscope will often enable one to identify the epithelial cells. The uric acid is determined by the murexid test and by the odor of hydrocyanic acid produced on heating.

The solution obtained by treatment with hydrochloric acid is to be tested as follows: A small portion is taken and tested for ammonia. The remainder is diluted, filtered, and rendered alkaline by ammonia. The filtrate is allowed to cool and is acidulated with acetic acid. There is thus obtained either a clear solution or one which is slightly cloudy and from which a white precipitate gradually separates out. The yellow-white flocculi which are present in the solution consist of ferric phosphate, which can be identified by filtering off the precipitate, dissolving the residue in hydrochloric acid, and treating with potassium ferrocyanide, which gives a blue precipitate with ferric salts.

The white insoluble residue is calcium oxalate. It sometimes shows characteristic crystals under the microscope, and can thus be identified. If a considerable quantity is present it should be collected and heated in a crucible to a red heat, when a mixture of lime and calcium carbonate will be formed. The residue shows, therefore, a strong alkaline reaction to moistened litmus paper, and when a drop of hydrochloric acid is added, bubbles of carbonic acid are given off. The filtrate, after the removal of the calcium oxalate crystals, may contain phosphoric acid, calcium, and magnesia.

1. Add some uranium acetate solution to a small portion of the filtrate, when if phosphorus is present a yellowish white precipitate of uranium phosphate may be produced.

2. Add ammonium oxalate. A white precipitate is evidence of the presence of calcium. The solution is warmed, the precipitate filtered off, and the filtrate rendered alkaline with ammonia. If crystals of ammonium-magnesium phosphate are produced, the presence of magnesium can be assumed.

## PARASITES IN THE URINE

The bacteria which may be found in the urine are very numerous, but the important species are the colon and typhoid bacilli, both of which may cause cystitis, staphylococci and streptococci and gonococci, and the tubercle bacillus. Actinomyces and the hooks from echinococcus cysts have been found very rarely. In Egypt the eggs of the Distomum hæmatobium are not rare in the urine, and in the tropics the embryos of the Filaria sanguinis are moderately common in the urine of those who suffer from the presence of these parasites in the blood.

The presence of typhoid bacilli in the urine of persons suffering from typhoid fever has recently attracted considerable interest, not because the presence of these bacteria incite either general symptoms or lesions in the genito-urinary tract, but because of the possibility that such urine may be a source of further infection, either through the hands, clothes, or drinking water. The typhoid bacilli have been found in approximately 25 per cent. of the cases, the figures varying a good deal in different epidemics. As a rule they are present only when a considerable quantity of albumin is passing through the kidneys. The bacteria appear in the urine from the second to the fourth week of the disease, and may persist for a considerable time during convalescence.

The gonococcus and the tubercle bacillus are the only species in which a morphological examination is of much value, though rarely one may find a urine containing a pure culture of the streptococcus pyogenes in which the morphology gives some clue to the germ.

The demonstration of the gonococcus in urine is not easy, and the results must always be controlled by the Gram method of staining, as diplococci can be found in all fluids which have passed through the urethra. It must be remembered also that in staining the gonococcus in albuminous urine, fixation at a high temperature may result in the cocci retaining the gentian violet, while in very acid urines organisms which are naturally Gram positive may decolorize.[1]

The urine should be centrifuged and the sediment examined by staining on the slide with the Gram stain, after fixing the sediment to the slide, as is directed under the heading of staining for tubercle bacilli. The other species of bacteria must be identified by cultural means.

Yeasts are not rare in urine containing sugar, and have no pathological significance, except that they may produce gas in the bladder and thus cause the patient to pass gas with the urine. The alcohol which they produce may also cause some confusion when testing urine for acetone.

### TUBERCLE BACILLI IN THE URINE

The question of the presence or absence of tubercle bacilli in the urine is of very great importance in the diagnosis of tuberculosis of the genito-urinary tract, inasmuch as the clinical symptoms are often not sufficiently well marked to warrant a diagnosis. The methods to be employed differ somewhat from those in use for staining tubercle bacilli in sputum, because of the presence in the urine, in a rather large proportion of cases, of bacilli which exactly resemble tubercle bacilli in their staining reactions when the ordinary carbol-fuchsin and acid decolorizers are used. These bacilli are the so-called smegma bacilli, which are constant inhabitants of the genital mucous membranes in both sexes, and are often found in urine passed from the bladder or even obtained by catheter. They occur most frequently in the region of the glans and vulva, but also on the skin, especially about the anus, between the toes, in the folds of the axilla and groin, and on the mucous membrane of the mouth. They belong to the same group as the bacilli which have been found in the exudate from the lungs in cases of gangrene.

To differentiate between the tubercle and smegma bacillus it is necessary to use special decolorizing agents which act upon the smegma bacillus and remove its color, without affecting the stain held by the tubercle bacillus.

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[1] *Libman:* Proceedings N. Y. Path. Society, N. S., vol. iii, 1904, p. 227.

One method is as follows:

1. Stain the dried and fixed sediment with carbol-fuchsin, heating gently until the dye steams, but do not boil.

2. Pour off the surplus dye and decolorize with 10 per cent. nitric or sulphuric acid.

3. Remove excess of color with strong alcohol.

4. Counterstain with aqueous methylene blue.

5. Dry and examine with an oil immersion lens. If any bacilli are found stained red, mark the spot by a circle of waterproof drawing ink, or determine the position by reading the coordinates of a mechanical stage and leave the slide in strong alcohol (97 per cent.) for eight hours. If at the end of that time the bacilli in this same field still retain their red color, they are tubercle and not smegma bacilli.

Another method is that recommended by Pappenheim, and depends for differentiation of the two organisms on the combined decolorizing effect of alcohol and rosolic acid (known also as corallin and aurin). The technique of the procedure has been given on page 392, but for convenience may be abstracted here.

1. Spread on a slide and fix as usual. Stain with hot carbol-fuchsin for two minutes.

2. Pour off the surplus dye without washing, and

3. Counterstain and decolorize by pouring over the slide three to five times the following solution:

| | |
|---|---|
| Strong alcohol | 100 |
| Corallin | 1 |

Saturate the above solution with methylene blue and add 20 parts of glycerine.

4. Wash off in water, remove surplus with blotting paper, and then dry in the air and examine.

The tubercle bacilli are stained red, the smegma bacilli blue.

Certain special procedures are necessary with urine which are not required in examining sputum. The urine should be as fresh as possible, as the alteration of the reaction changes somewhat the color reactions of the bacilli. It is usually necessary to centrifugate a considerable quantity of the urine, and, if large amounts of thick nucleo-albumin and pus are present, to use some method of softening it so that the bacilli may be deposited. The best is that of Biedert, as given on page 394.

After the bacilli have been collected in the centrifuge tube they should be mixed with a little blood serum or egg albumin, so that when the slide is stained the bacilli will not be removed by the aqueous fluids. It is advisable to employ albumin in fixing all kinds of urinary deposits to the slide as a preliminary to staining, as otherwise they often wash off from the slide during the necessary manipulations.

In all important cases, especially before undertaking operative procedures, it is advisable to resort to animal inoculations or to inject tuberculin and not to depend upon the results of stains. Large quantities of urine may be injected into the abdomen of the guinea-pig, and in cases where there is but little pus this may be preferable, as there is less chance of missing a possible bacillus in so large a quantity as 10 c.c.; but several animals should always be injected with the same specimen, as there is considerable danger of the animal dying of general peritonitis before any tuberculous lesions can develop. When a considerable amount of thick pus can be obtained it should be inserted in the groin. When several are injected one is likely to survive the infection and go on to die of tuberculosis should the bacilli be present in the injected fluid.

## TUBERCULIN AS A MEANS OF DISCOVERING GENITO-URINARY TUBERCULOSIS

The use of tuberculin injections to determine the presence or absence of a tuberculous lesion has recently come widely into use. The technique is simple. The normal temperature variations of the patient are first determined for twenty-four hours, preferably by observations every two or three hours. After the injection of the tuberculin the temperature must be taken at least every two hours or a slight reaction may be overlooked.

The old Koch's tuberculin is to be used, which is a glycerin extract of the bodies of the tubercle bacilli, and not the newer preparations, TR, etc.

One-half to one milligram of the tuberculin is to be injected, the smaller quantity in children, the larger for adults. These quantities can be easily measured by properly diluting the original strong dilution.

The injection should be made into the deeper tissues with a hypodermic syringe which has been sterilized by boiling. If after this first injection a rise of temperature of 0.5° C. is observed, the diagnosis of a tuberculous lesion is probable, though a good reaction, according to some observers, requires a rise of at least 1.2° C.[1] This rise regularly occurs within the first thirty-six hours after the injection, usually within four or five hours.

If no rise is observed, a second or even a third injection may be given with a dose not to exceed 10 milligrams; anything above this will cause a rise of temperature in healthy adults. A positive result is a very strong evidence of an active tuberculous lesion in the body; a negative result is not quite so valuable, as in a certain number of cases of old encapsulated tuberculous lesions, notably in tuberculous peritonitis, a positive reaction does not occur, even with considerable doses. This is probably due to the poor circulation in such nodules, which prevents the escape of the toxins of the bacilli and equally the entry of the tuberculin. The reaction has been noted in cases of lepra and also in actinomycosis, but these two conditions can as a rule be easily differentiated from tuberculosis by the clinical symptoms and demonstration of the specific germ.

The careful use of well-prepared tuberculin is not usually dangerous. It contains no tubercle bacilli. Some observers, however, have noted that the pulmonary symptoms in phthisis have been aggravated by the injection, and cases of serious collapse after the injection, while rare, have occurred. It is well, therefore, to limit the use of this powerful agent to such cases as need an immediate diagnosis either as a preliminary to operative interference or to climatic treatment.

## CHANGES IN THE URINE IN DISEASES OF THE GENITO-URINARY TRACT

**Cystitis.**—In cystitis of a moderate grade the urine is usually slightly acid and free from albumin. There is a slight diffuse cloud which contains only a moderate number of leucocytes and a few bladder cells. In more severe cases the reaction is usually alkaline to litmus or becomes so soon after passing, and the amount of deposit is considerable. A trace of albumin is present.

In advanced cases of acute cystitis, the urine is dark from the presence of altered blood pigment, and the leucocytes are usually softened down to a stringy mass which may even be so thick as to make the urine difficult to pour from one vessel into another. In the thick sediment may be found large numbers of triple phosphate crystals and bacteria of all sorts. In uncomplicated tuberculous cystitis the amount of pus is not very great and the urine is usually acid in reaction.

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[1] *Zupnik:* Deut. Arch. f. klin. Med., Bd. lxxxvi, 1903, p. 297.

An acute membranous cystitis is occasionally seen in which more or less complete casts of the bladder may be passed. These casts are composed of necrotic epithelial cells, fibrin, pus, and bacteria.

**Gonorrhœa in the Male.**—In the acute stages in the male the thick, creamy pus which can be expressed from the meatus by pressure along the course of the urethra, is composed of large numbers of neutrophilic pus cells, and mingled with them an abundance of eosinophile cells. The cells are not all polynuclear, but the mononuclear appearance is due most often to the nucleus of the polynuclears collecting at the center of the cell and not remaining distributed throughout the cell body, as is the case in the circulating blood. The biscuit-shaped pairs of the gonococci can be seen in the bodies of the leucocytes, and occasionally in the bodies of the large epithelial cells which have desquamated from the surface of the urethra.

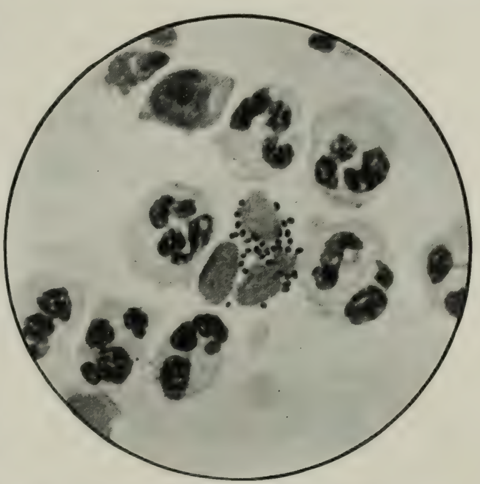

Fig. 168.—Gonococci from Urethral Pus. Stained by the Jenner method. Magnified 1,200 diameters.

The smears of the pus can best be made by spreading with the edge of another slide just as in smearing blood.

If the discharge is not abundant or only present as a morning drop, the patient should be given two glass slides with the instruction to collect any discharge between the two slides, fasten them together by a rubber band and bring them for examination. The slides are then separated and a fair smear of the pus will be found on each.

The simplest and most convenient stain for the routine examination of gonorrhœal discharges is the Jenner stain, which fixes and stains at the same time. But inasmuch as there are diplococci in the urethra which are morphologically so similar to the gonococcus that a differentiation is impossible, it is necessary in doubtful cases to resort to a differential stain. This is especially true of chronic gonorrhœas of long standing, in which a secondary infection, or at least the growth of other bacteria, takes place. The stain which is used to distinguish between the gonococcus and the various cocci is that bearing the name of Gram. The details of the stain will be given under Exudates (page 640).

A very few cases have been reported in which a patient suffered from a urethritis, the discharge from which contained diplococci which closely resembled the gonococcus in morphology and decolorized with Gram, and yet were not gonococci, as was shown by cultural tests. Nevertheless these cases are so extremely rare that under ordinary conditions such a possibility may be neglected, and biscuit-shaped diplococci found in pus cells from a vaginal or urethral discharge and decolorizing with Gram may be considered as gonococci. The only cocci which at all resemble the gonococcus in their morphology and reaction to Gram are the meningococcus intracellularis of Weichselbaum, found in the pus from the meninges of cases of epidemic cerebro-spinal meningitis, and the micrococcus catarrhalis. Both of these organisms differ in their cultural characteristics from the gonococcus. The micrococcus catarrhalis does however incite a subacute or chronic urethral discharge of the mucoid character and without many pus cells. These cases are rare, and can only be positively distinguished from true gonorrhœa by cultivating the organisms.

As an attack of gonorrhœa becomes chronic the appearance of the discharge changes from the thick, creamy pus of the earlier stages to a thin, stringy fluid containing fewer pus cells and more mucus and epithelium. The gonococci also become fewer in number, and may entirely disappear from the penile discharge, which is often only present in the morning as a morning drop. If, however, the urine of such a case be collected and carefully examined, long threads will be found in it, and these threads frequently contain the gonococcus.

The threads are of two varieties, one composed chiefly of mucus covered with a moderate amount of epithelium, and the other, as a rule, thicker, shorter, and more opaque, and composed very largely of pus cells. Very short, comma-like threads are also seen, which are derived from the glands of the urethra and the prostate, and covered largely with cylindrical epithelium, which often shows hyaline degeneration. The exact site of origin of these threads may be determined by having the patient pass his urine into two glasses; the threads in the first will be washed out of the anterior urethra by the stream of urine, while the urine in the second glass will contain the threads from the posterior urethra and the prostate.

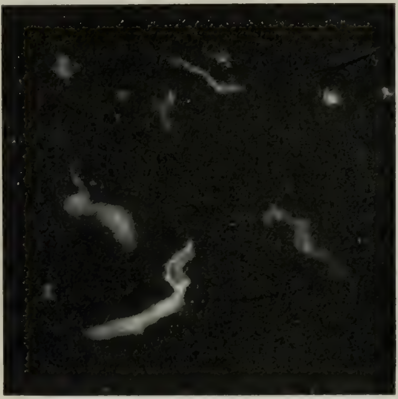

Fig. 169.—Urethral Threads.
Twice natural size.

Another method of obtaining the prostatic threads is to massage that organ through the rectum after the patient has passed a little urine, and then, after the massage, to have him wash out the prostatic threads by means of the remaining urine. The careful examination of these threads is of the greatest importance in deciding whether a case of chronic gonorrhœa is cured or not. The gonococci often persist in the glands of the urethra when they have disappeared from the mucopurulent fluid which can be obtained from the meatus.

The presence of mucous threads in the urine is, however, no proof of the existence or previous existence of a gonorrhœa. They may be seen in moderate numbers in the urine of persons who have never suffered from that disease, but who from sexual excess or masturbation have kept up a chronic congestion of the urethra. The threads in these non-infectious cases may contain a few bacilli or cocci, which are positive to Gram, and are merely accidental inhabitants of the urethra.

**Gonorrhœa in the Female.**—The localization of the germ in acute gonorrhœa in the female varies a good deal, depending upon the age of the patient. The acute gonorrhœa of infants is usually in the conjunctiva, while that of small girls is usually in the vagina, and that of adults, in the majority of cases, either in the urethra, the cervix, or the vulvo-vaginal glands of Bartholin.

In chronic cases in adults the vulvo-vaginal glands are infected in a large proportion of the cases. In the adult the infection frequently invades the uterus and the tubes, and the gonococcus may be obtained in pure culture from these situations. The diagnosis of gonorrhœa from stained specimens is more difficult in the female than in the male, and especially in the chronic cases, and the precautions to avoid error by the use of the Gram stain are even more important, because of the large flora of the female genital tract.

In the vulvo-vaginitis of small girls the presence of the gonococcus is very easy to determine, as a rule, in the abundant purulent discharge from the vagina.

In adults, gonorrhœal vaginitis is very rare. Pus for examination in chronic cases should be obtained from the cervix or urethra by means of a sterile platinum loop or by a small sterile swab of cotton, and the resulting fluid smeared on a slide, fixed and stained by Gram's method.

**Chronic Prostatitis.**—The amount of deposit which may be obtained after the massage of the normal prostate and the washing out of the urethra by the urine is very small, and consists chiefly of epithelium, strings of mucus, and a few amyloid bodies and spermatozoa expressed from the seminal vesicles. The amount which may be obtained from a case of chronic prostatitis is much larger, and can often be obtained from the meatus after massage without any mixture of urine. When examined microscopically this fluid will be found to contain, besides many epithelial cells, large masses of hyaline material and amyloid bodies from the prostate, lecithin granules, spermatozoa, and finally the so-called " Böttcher's " crystals. These are needle or whetstone shaped crystals which are peculiar to the prostatic fluid and give it its characteristic seminal odor. These crystals are phosphates of an organic base and can not usually be demonstrated in the prostatic sediment when obtained from the urine, but only when in pure form as expressed from the meatus after prostatic massage. They are soluble in alkalies and formaldehyde, which distinguishes them from the Charcot-Leyden crystals. Their formation is hastened by the addition of a few drops of a 1 per cent. solution of ammonium phosphate and allowing the mixture to dry slowly under a cover glass.

A reaction which is useful in determining the presence of spermatic fluid is as follows:

**Florence Reaction.**[1]—The reagent consists of a mixture of iodine, 2.54; potassium iodide, 1.65; distilled water, 30. The iodine should be washed with distilled water before dissolving.

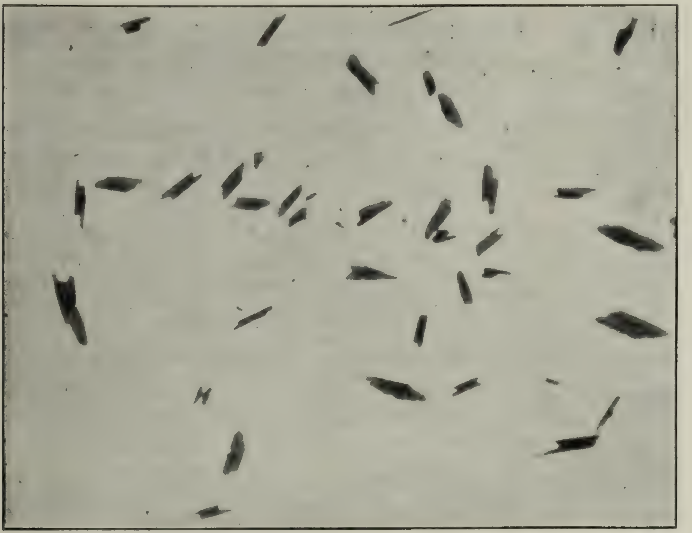

Fig. 170.—Crystals Obtained in Spermatic Fluid with Florence's Reagent.

The suspected stain is cut or scraped off the cloth or tissue to which it is attached and placed on a slide. A few drops of the reagent are added and the preparation promptly examined with a medium power lens. The crystals which are formed are single lance-shaped bodies or rosettes of the same; also double lance-shaped or pyramidal forms, and finally, small rhombic crystals. All are of a dark brown color.

The reaction is given very promptly by human semen; also by that of animals, though less characteristically, and finally by extracts of organs, especially the liver and brain, crushed insects, etc., and even by fresh extracts from vegetable seeds, but not by uterine or vaginal secretion nor by that from the nose or throat. Bocarius[1] has shown that the crystals are due to cholin which is contained in the seminal fluid and also in organic extracts. The reaction can be obtained from traces of human semen whether it be fresh or has been dried. Specimens twenty-two years old have given the reaction, and even putrefaction of the semen does not prevent a positive reaction. The value of the test is therefore dependent upon the proof that such substances as give the reaction have not been mixed with the stain to be tested.

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[1] Arch. d'Anthropologie, tome x and xi, 1896.

## V. FUNCTIONAL EFFICIENCY OF THE KIDNEYS

The exact determination of the functional efficiency of one kidney or of both kidneys acting together is of interest from two points of view: first, because of the great importance of these organs as the channel through which the excretion of the nitrogenous substances, salts, and fluids of the body largely takes place, and the marked clinical symptoms which arise when this excretion is interfered with; and, second, because one healthy kidney is capable of taking over the work of the other, thus permitting the surgical removal of the diseased organ.

The procedures which we employ for this purpose are of two types:

1. We may determine whether the two kidneys taken together are sufficient for the needs of the body metabolism. The result of such a determination does not, however, furnish any hint of the individual capacity of the two organs—in other words, does not tell us whether the kidney which is to be left in situ possesses sufficient secretory capacity to carry out the work of the body in removing waste products.

2. We may determine the efficiency of each kidney and thus discover whether the organ which is to remain in the body is capable of successfully carrying on its own work and also that of the diseased kidney which is to be incised or removed.

3. By chemical or physical examination of the blood, we may determine the presence or absence in that fluid of an excess of metabolic products which under normal conditions are excreted by the kidneys and in certain types of disease are retained in the

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[1] Zeit. f. phys. Chemie, Bd. xxxiv, 1902, p. 339.

blood by the impermeability of the kidney filter. This method furnishes no information concerning the relative condition of either kidney, except that both together are sufficient or insufficient.

## DETERMINATION OF EFFICIENCY BY THE EXAMINATION OF MIXED URINE FROM BOTH KIDNEYS

The method by which the determination first mentioned is obtained is usually based upon an examination of the combined urine as it is passed from the bladder, and is the conventional microscopical and chemical procedure used in clinical medicine for the determination of the presence of abnormal constituents or a reduction in the excretion of the normal substances.

The presence of albumin and casts in a mixed urine, for example, furnishes us with an evidence of kidney insufficiency and also signifies a lesion of one or both kidneys, yet an accurate estimate of the efficiency can not be made from mere qualitative determinations. For example, a tuberculous kidney may permit the transudation of a large amount of albumin and the formation of numerous casts, and yet the remainder of the kidney substance, which is not affected by the tuberculous process, may be quite sufficient to excrete an abundance of urine and that of good quality, so that if the urine from this kidney is separately collected and examined it will be found that it has nearly the same specific gravity, and is the same in quantity as that produced by the other and healthy organ. Clinical instances of this fact are seen when one tuberculous organ has been removed, and the other, though slightly involved by the same infection, is found to be perfectly able to carry on the work of removing the metabolic products and to allow the patient to resume his vocation.

An illustration of exactly the opposite type is seen in advanced chronic nephritis where the urine may contain only an occasional cast and a trace of albumin and yet the kidney efficiency be reduced to the very lowest limit consistent with the continuance of the patient in an apparently normal condition, while the slightest additional strain thrown upon the kidneys, such as the taking of an anæsthetic, will precipitate an acute exacerbation of the disease or an attack of uræmia. The casts and the albumin evidently furnish no accurate quantitative measure of the efficiency of the kidneys.

It is possible also that although the urine contain albumin and numerous casts, these evidences of renal disease may be partly or even wholly derived from one kidney, the other organ being in a fairly healthy condition. An examination of the mixed urine, therefore, throws no light upon the condition of either kidney, but only upon both taken together.

### SPECIAL METHODS USING MIXED URINE

**Nitrogenous Excretion through the Kidneys.**—One sees in the literature continued references to the fact that the determination of the amount of nitrogen excreted will enable one to judge of the conditions of the kidneys. A diminution in the normal excretion is said to point to a lesion of the renal tissue. It is first necessary to decide what must be considered as a normal excretion, first, of urea, and second, of the total nitrogen.

The results of careful analyses made of the urine of patients who are on a measured and constant diet have shown that the amount of urea and the amount of nitrogen excreted depends largely upon the composition and amount of the food, and upon other conditions—for example, the amount of fluid taken, the amount of exercise, and the bodily weight of the patient. The important thing to note is that there is no fixed quantity of urea or nitrogen excreted.

The human body excretes either exactly as much as it takes in, or, in the case of febrile conditions, wasting diseases, and diabetes, more than it takes in, for the reason that there is a breaking down of the proteid tissues of the body; or, finally, it excretes less than it takes in. This retention of nitrogen is noted in healthy persons where the body is storing away proteid and gaining weight. In such conditions the nitrogen is stored, not excreted, and the urea excretion may be low as compared with the amount of urea which can be derived from the proteid taken in in the food. It must be remembered that normal people do not excrete urea in a constant way, but retain a certain amount for a few days, then excrete the excess, then accumulate again. It must also be remembered that a variable amount of the nitrogen of the body is excreted in the fæces, and without careful analysis of this excretion it is impossible to judge of the total nitrogen removed from the body.

It has been shown by careful analyses in cases suffering from various forms of nephritis that there may be nitrogen retention in these cases, but that there may be also periods of excessive elimination, so that a single examination does not furnish any clue as to the occurrence of nitrogen retention. The type of the disease is also of importance, for in uncomplicated cases of chronic parenchymatous nephritis there is usually no nitrogen retention, while in interstitial nephritis there is often a marked retention of the nitrogenous substances, though not until the later stages of the disease.

All analyses without previous determination of the nitrogenous intake are quite useless, for the amount of fluctuation even in nephritics may be no more than appears in normal persons.[1]

**Excretion of Chlorides by the Kidneys.**—The results of the analysis of the chloride excretion in cases of health and disease have not furnished us with facts of value in diagnosing the permeability of the kidneys. C. v. Noorden [2] has not noted a retention. Widal has found a marked retention in cases of nephritis with œdema. Other observers have obtained variable figures. (See also page 424.)

**The Determination of the Freezing Point of the Urine.**—This method, which gives us an accurate means of determining the total molecular excretion of the kidneys, is possibly of more value than the analysis of the excretion of a single constituent of the urine, and the results can be obtained more quickly than by purely chemical means. But the freezing point varies so greatly even in health, or in diseases other than nephritis, often depending upon the amount of food and water taken by the patient, that the low freezing points occasionally seen in the urine of nephritis can not be relied upon as furnishing definite diagnostic hints.

**The Excretion of Substances Artificially Introduced into the Body.**—The first of these substances is methylene blue, which is excreted by the kidneys partly in the form of a colorless chromogen which must be heated with acetic acid in order to bring back its blue color, and partly in the form of the original substance.

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[1] For further details see *Kohler:* Arch. f. klin. Med., 1900, Bd. lxv, p. 542; *Casper* u. *Richter:* Funktionelle Nierendiagnostik, Berlin, 1901; *v. Noorden* u. *Ritter:* Zeit. f. klin. Med., 1891, Bd. xix, p. 197; *Strauss:* Die chron. Nierenentzündungen, Berlin, 1902; *v. Koziczkowsky:* Zeit. f. klin. Med., 1903, Bd. li, p. 287.

[2] Sammlung klin. Abhandlungen, Heft 2, Berlin, 1902.

It has been found that in cases of chronic interstitial nephritis after the injection of 0.05 grams of the medicinal preparation of the dye the excretion of the methylene blue is delayed for a very considerable time, even as long as three hours. In parenchymatous nephritis there is no alteration in the time of excretion. Some cases, however, of advanced interstitial nephritis have been found to exist which excreted methylene blue in the same quantity and in the same time as normal persons[1]; that is, in about 15 tc 30 minutes.

Indigo-carmine has also been suggested for use in testing the efficiency of the kidney,[2] especially in connection with cystoscopic examination of the mouths of the ureters. If after an intramuscular injection of 0.05 grams of this substance the stream from the ureter of one side is colored before that of the other, it has been assumed that a lesion of the renal tissues exists on the side on which the excretion has been delayed. If no blue color appears on one side, it is justifiable to conclude either that the kidney of that side is absent, that the ureter is completely blocked, or that the kidney substance is so completely destroyed by disease that no urine can be produced. The procedure, however, is too new to permit of thorough investigation, and while some surgeons have obtained satisfactory results the method has not escaped sharp criticism.[3]

## DETERMINATION OF EFFICIENCY BY EXAMINATION OF URINE FROM EACH KIDNEY

The determination of the efficiency of the individual kidney depends naturally upon the possibility of collecting the secretion of each kidney separately and during exactly the same period of time. Then, as each organ is elaborating and excreting the products of metabolism of the circulating blood which furnishes each kidney with a fluid of exactly the same physical and chemical qualities, any difference in the urine secreted by either one will furnish a clue to the relative amount of work which each is capable of performing.

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[1] See *Achard* et *Castaigne:* L'Exam. clin. d. fonctions rénales, L'œuvre médico-chirurgical, Paris, 1900; *Landau:* Zeit. f. klin. Med., 1902, Bd. xlvi, p. 210.

[2] *Voelcker* u. *Joseph:* Münch. med. Woch., 1903, p. 2081.

[3] *Karo:* Monatsbericht f. Urol., Bd. ix, 1904, p. 1.

The means of obtaining urine from each kidney under fairly normal conditions are the separate collection of the urine from each ureter by one of the so-called segregators or by direct catheterization of the ureters. If both ureters can not be catheterized for any reason, the catheter can be placed in one and the urine from the other kidney allowed to collect in the bladder and withdrawn by a second catheter. When working, however, with specimens of urine obtained under these conditions minor variations in composition are of much less importance than when each ureter can be catheterized.

The condition of the most importance, which must always be fulfilled, is that the urine be collected simultaneously from each kidney and for an exactly equal period of time. In persons with normal kidneys the quantity, chemical and physical properties of the urine from each kidney are approximately the same.

The technical difficulties of both procedures unfortunately greatly limit the usefulness of this method. Not every surgeon can use a cystoscope or a segregator, and it may even be wise not to submit a patient who is seriously ill to the shock incident to so prolonged an anæsthesia as may be necessary in order to collect sufficient quantities of urine for analysis.

Even if the urine can be obtained without placing the patient under an anæsthetic, the reflex irritation may give rise to suppression of urine or may set up circulatory changes in the renal secretion which interfere with the tests. For example, a reflex polyuria has been noted from one kidney after insertion of a catheter in the corresponding ureter and also a suppression from the same cause. These phenomena have led Albarran[1] to the conclusion that in order to avoid the effects of reflex irritation in altering the composition of the urine the fluid must be collected for a long time—at least two hours. This is not often possible, and allowance must be made for slight chemical differences.

The course of the examination of specimens of urine obtained by ureteral catheterization is as follows:

The urine from each kidney is preferably collected in sterilized graduated conical centrifuge tubes holding 15 c.c. These are closed by sterile corks and immediately centrifugalized. The clear supernatant urine is then poured off, its reaction obtained, and its specific gravity determined. This usually must be done either by means of the Westphal balance, or better by a small pycnometer flask which is filled and weighed on a balance. The Saxe hydrometer may also be used. As the specific gravity of the urine is only of value when compared with that of the other side, the results need not be corrected for temperature, if both determinations are made within a few minutes of each other.

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[1] C. R. de. l'Acad., 1903, tome cxxxvi, p. 1207. See also *Gobell:* Münch. med. Woch., 1903, p. 1993.

The clear supernatant fluid is poured off from the deposit in the tip of the centrifuge tubes and the freezing point is determined. To do this with the Beckmann apparatus requires about 15 c.c. of urine and twenty minutes' time. If the amount of urine is less than 10 c.c. from one side or both, it must be diluted carefully and the results multiplied accordingly. The values so obtained are accurate enough for clinical purposes. If the freezing points agree closely—that is, to within one or two figures of the second decimal—and are both more than 1° C. below that of distilled water, the urines may be assumed to be normal, provided the microscopical examination shows that no casts, albumin, or sugar are present. If the freezing point of one urine is very different from that of the other side, an insufficiency of the corresponding kidney is very probable. Sometimes this is temporary, as has been observed in renal colic, when the urine regains its normal character after the passing of the attack or after the removal of the stone. Where the kidney is the site of a serious lesion, however, the difference is constant and usually very marked; the normal side may show a depression of 1.5° C., the diseased side a depression of only 0.10° to 0.80° C. The difference between the freezing point of the urine from each kidney is marked in tumors of one kidney and in kidneys in which much tissue has been invaded by either tuberculous disease or suppuration.

The difference is often very great with a one-sided hydronephrosis; but care must be taken first to drain the urine which has collected in the hydronephrotic sac, and then to obtain the excretion of the kidney direct and during a given period, for the urine in a sac or dilated pelvis may vary greatly from that directly excreted by the kidneys. If the freezing point of the urine from both sides is less than 1° C. below that of water, both kidneys are probably insufficient and the $\delta$ of the blood should be determined. As a rare occurrence such a slight depression of the freezing point may be observed after extreme diuresis from any cause, but this can be easily excluded by the history.

After the freezing point determination has been made the urine from each side should be carefully tested for albumin and sugar by the usual methods. It must be remembered in this connection that traces of albumin may appear in any urine during the taking of an anæsthetic, and though this is not invariable, yet it is present sufficiently often to detract somewhat from the results of the more delicate tests. It is better, therefore, if possible, not to give an anæsthetic if the patient can be catheterized without.

A trace of albumin may also appear in the urine if the operator in any way injures the mucous membrane of the ureter; or, when the urine is allowed to flow into the bladder from one kidney, by any injury to the bladder sufficient to cause a flow of blood. If the freezing point determination has been made, quantitative determination of the chlorides or urea is not absolutely necessary, though a very considerable variation in the quantity of these substances may be noted in urines with approximately the same freezing point.

The deposit at the tip of the centrifuge is to be carefully examined microscopically, especially for casts and bacteria. The casts give positive evidence of a kidney lesion; the bacteria may furnish a clue to the cause of the kidney lesion.

The only morphological diagnosis which is allowable is unfortunately confined to two species, the tubercle bacillus and the gonococcus. The precautions for the decolorization of the smegma bacilli should always be taken, and the gonococcus must decolorize by Gram. Pus corpuscles give valuable evidence; crystals and epithelium are of little importance from a diagnostic aspect.

Another method of obtaining a judgment upon the relative excretory efficiency of the two kidneys is by producing an artificial glycosuria and determining the amount of sugar excreted by each kidney. About half an hour before the catheterization is to be done, 5 mgm. of phlorizin dissolved in warm water or dilute sodium carbonate solution are injected subcutaneously. This amount is sufficient to produce a transitory glycosuria lasting for about three hours in a person with normal kidneys, and without danger to the patient. The action of the drug is presumed to be upon the parenchyma of the kidney and alters the cells so that the kidney, it is supposed, excretes the sugar normally present in the blood. If the amount of functionally capable parenchyma is in any way reduced, either by a nephritis or by an inflammation of the kidney substance or by compression by a tumor, then the amount of sugar appearing in the urine will either be greatly reduced or in severe cases no sugar at all will appear. The relative amounts of sugar in the urine from the two sides is easily determined quantitatively, either by titration or by fermentation in a suitable apparatus. The results agree very closely with those obtained by the freezing point method. If the appearance of the sugar in the urine is delayed for an hour or so from either one or both kidneys, a general lesion of the kidney parenchyma may be suspected.

### DETERMINATION OF EFFICIENCY BY THE FREEZING POINT OF THE BLOOD

We owe chiefly to Korányi[1] the enunciation of the fact that the molecular concentration of the blood is exceedingly constant, the depression in the freezing point being .55° to .57° C. below that of distilled water. It has also been shown by the same observer that in cases of nephritis there is often a retention of molecules in the blood, as evidenced by an increased depression of the freezing point, which may reach even to −.75° or −1° C. The reason for this retention is the impermeability of the kidney tissue for the products of metabolic activity. While it has been shown that in many cases of nephritis the freezing point is depressed below the normal, yet this phenomenon is not constant nor does it appear early in the disease, nor when the lesion is unilateral. A unilateral lesion is practically not met with in the ordinary forms of acute or chronic nephritis, but when the kidney is the site of a tumor or stone, or is subject to other forms of irritation, a nephritis may be incited in one organ and not in the other.

A great depression in the freezing point indicates that the kidneys are not sufficiently active to carry off the surplus molecules, and no operative procedures of any magnitude should be undertaken on such a case. A number of surgeons[1] have found that if the freezing point is much below —.60° C. or —.62° C., a nephrectomy is likely to be followed by death from uræmia or suppression of urine, and yet no absolute line can be fixed beyond which no patient should be subjected to operative interference. For example, Barth[2] reports a successful operation upon a patient whose blood froze at —.62° C. The kidney removed was atrophic, the other normal. Israel[3] also has operated with success upon patients with a $\delta$ of —.60° C. It is evident that the final decision depends much upon the skill of the operator and more upon the nature and extent of the proposed operation. An incision, for example, into a pus kidney should not be omitted simply because the patient's blood freezes at —.70° C., nor should a prolonged and difficult operation be undertaken if the freezing point is —.60° or —.62° C. A good many nephrectomies have been carried out with marked benefit to the patient's condition though the freezing point of the blood was below —.56° C.

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[1] An excellent résumé of the subject is contained in a short monograph by *v. Korányi* on "Die Wissenschaftlichen Grundlagen der Kryoskopie," Berlin, 1904. See also *Strauss:* Bedeutung d. Kryoskopie f. d. Diag. u. Therap. von Nierenerkrankungen, Berlin, 1904.

The choice of the anæsthetic is also important, and it should be remembered that ether and chloroform both irritate the kidneys, so that nitrous oxide should be used, if possible.

A moderate depression of the freezing point has been seen in the blood of persons suffering from chronic heart disease, from renal colic due to calculi, with large abdominal tumors, and occasionally[4] in the cachexia dependent upon the presence of malignant new growths.

The same phenomenon has been observed in diabetes, in lead-poisoning, and also during an attack of acute gout. It is evident, however, that in the three conditions last mentioned a renal lesion might exist without other symptoms such as casts or albumin and thus account for the lowered freezing point.

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[1] *Kümmell* u. *Rumpel:* Beit. z. klin. Chir., 1903, Bd. xxxvii, p. 788 ; *Rumpel:* Münch. med. Woch., 1903, p. 19 ; *Tinker:* Johns Hopkins Hosp. Bull., 1903, vol. xiv, p. 162.

[2] Verh. d. deutschen Gesellschaft f. Chirurgie, 1903, p. 343.

[3] Mitteil. a. d. Grenzgebt. d. Med. u. Chir., 1903, Bd. xi, p. 171.

[4] *Engel* (Berl. klin. Woch., 1904, p. 828) examined thirteen cases of malignant growth and found no depression below —.58° C., while *Engelmann* (Mitteil. a. d. Grenzgebt. d. Med. u. Chir., 1903, Bd. xii, p. 396) found a marked depression in five out of ten cases examined, though the tumors in some of the five cases may have exerted pressure on the ureters and thus set up changes in the kidney.

While, therefore, a low freezing point does not necessarily indicate nephritis with retention, neither does a normal freezing point of the blood indicate a normal condition of the kidneys. A very considerable proportion of cases of uræmia show practically normal values for the $\delta$ of the blood.

An important observation bearing on this point has been recorded by Stockman,[1] who saw a normal $\delta$ shortly before death in a person with advanced tuberculosis of one kidney and absence of the other. Evidently the small portion of the kidney which was not invaded by the disease was sufficient to remove the surplus molecules from the blood.

It is evident, then, without presenting further data on the subject, that while the determination of the freezing point may frequently be of great value in the diagnosis of a renal insufficiency, the method does not give results which can be blindly followed as a guide, without taking into consideration many other factors, among which the operative facility of the surgeon and the nature of the proposed operation are not the most unimportant.[2]

The determination of the freezing point of the blood and other methods of estimating the functional capacity of the kidneys have not furnished us with any means of diagnosis or prognosis with regard to puerperal eclampsia. Indeed, most observers report that the freezing point of the blood is normal or is high—that is, approaches the freezing point of distilled water. This is quite in accord with the recent investigations of the pathological alterations underlying puerperal eclampsia, which have shown that the changes in the kidney are slight and relatively unimportant, while the chief alterations have been found in the liver. It is of course possible, however, to have a puerperal eclampsia and a coexistent nephritis, but many cases are seen in which the renal symptoms are unimportant.

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[1] Monatsbericht für Urologie, 1902, Bd. vii. p. 583.

[2] Reports of cases and results may be found in *Kövesi:* Wien. klin. Woch., 1904, p. 879; *Van der Poel:* N. Y. Medical Journal, 1904, vol. lxxix, p. 721; *Tilden Brown:* Med. and Surg. Reports of Presbyterian Hospital, vol. v, New York, 1902; *Krotoszyner* and *Willard:* Amer. Jour. Med. Science, 1904, vol. cxxvii, p. 821; *Watson* and *Bailey:* Bost. Med. and Surg. Jour., 1902, vol. cxlvii, p. 609; *Kümmell:* Arch. f. klin. Chir.. 1903. Bd. lxxii, p. 1.

# PART VIII

# TRANSUDATES AND EXUDATES

## I. GENERAL CONSIDERATIONS

THE examination of transudates and exudates may furnish facts of diagnostic value in three ways: the study of the morphological elements contained in them, the identification of the bacteria which may be present, and, more rarely, from a study of their physical properties.

### TRANSUDATES

Transudates are not due to inflammation, but to disturbances in the circulation of the blood or changes in the osmotic relations between the blood and the tissues. They are usually transparent, light yellow fluids with a faint greenish tinge. They frequently deposit a slight, flocculent coagulum on standing and have an alkaline reaction.

In examining these fluids the specific gravity should be determined. It is lower in simple transudates, such as occur in the case of a nephritis, than in truly inflammatory exudates, such as occur in acute pleurisy. The average specific gravity is as follows:

In hydrothorax, lower than 1.015.
In ascites, lower than 1.012.
In anasarca, lower than 1.010.
In hydrocephalus, lower than 1.008.
In pleurisy, between 1.017 and 1.027.
In peritonitis, between 1.016 and 1.022.

Since the specific gravity is dependent chiefly upon the albumin content of the fluid, we can roughly approximate the amount from the specific gravity. The pleural exudates contain rarely less than 4.5 per cent.; those from the peritoneum, from 2 per cent.

to 2.5. In transudates from the pleural cavity it is always under 2.5 per cent.; in transudates in the peritoneum, between 1.5 per cent. and 2 per cent.

### DETERMINATION OF PROTEID CONTENTS

The determination of the total albumin present in an exudate is most accurately accomplished by coagulating the fluid after acidulation with acetic acid and weighing the dried coagulum as is described in detail on page 503. This procedure, however, is somewhat complicated for clinical purposes and the practitioner may prefer the Esbach tube.

The transudate should be considerably diluted before adding the reagent. It is generally necessary to add about 9 volumes of water to 1 volume of the fluid. This gives the total proteid present in the fluid, or A.

In order to obtain the proportions of albumin and globulins present, it is necessary to precipitate the neutral fluid with a saturated solution of ammonium sulphate.

B. The fibrinogen is precipitated by adding to 7.2 parts of the fluid 2.8 parts of ammonium sulphate solution.

C. A large proportion of the euglobulin is precipitated by adding 3.6 parts of ammonium sulphate solution to 6.4 parts of the transudate.

D. The pseudoglobulin is precipitated by the addition of an equal bulk of ammonium sulphate solution.

After the precipitate has formed, the mixtures may be filtered and the moist filter steamed in an Arnold sterilizer until the proteid is coagulated. The excess of ammonium sulphate is removed by washing with hot distilled water and the filters are dried and weighed, or the nitrogen of the coagulum and filter may be determined by Kjeldahl and the proteid determined by multiplying by 6.25. For clinical purposes the Esbach tube may be used,[1] by adding to the mark U a sufficient quantity of the exudate and the proper proportion of the ammonium sulphate. After the proteids have settled to the bottom of the Esbach tube the supernatant fluid should be removed by a pipette and the heavy ammonium sulphate solution replaced with distilled water to the mark U. The upper portion of the tube is then filled with the Esbach reagent, a little acetic acid added, and the whole is inverted several times to mix the fluids thoroughly and set aside for 24 hours.

B gives the fibrinogen content of the exudate.

C minus B gives the euglobulin content of the exudate.

D minus C gives the pseudoglobulin content of the exudate.

A minus D gives the albumin content of the exudate.

This method may also be employed in determining the relations of the proteids in the urine and the blood.

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[1] *Oswald:* Münch. med. Woch., 1904, p. 1514.

The proportions between the different kinds of proteids, both in the serum of the blood and in transudates and exudates, vary considerably. As yet we do not know the reason for such variations nor the laws under which they occur. The table[1] which follows may, however, be of some interest in this connection.

| | | Euglobulin | Pseudoglobulin | Albumin |
|---|---|---|---|---|
| Chest: | Pleuritis | 19.7 | 27.2 | 53.1 |
| | Cardiac hydrothorax | 14.8 | 26.3 | 58.8 |
| Abdomen: | Cirrhosis | 20.5 | 37.2 | 43.2 |
| | Cardiac transudate | 20.8 | 26.3 | 52.8 |
| | Ascites from carcinoma | 12.8 | 22.2 | 64.9 |
| | Tuberculous peritonitis | 25.5 | 36.9 | 37.4 |
| | Tuberculous peritonitis | 23.9 | 13.8 | 62.3 |
| Blood serum: | Placental blood | 13.1 | 20.97 | 66.0 |
| | Umbilical vein blood | 17.0 | 13.0 | 69.8 |
| | Blood from case of myocarditis | 26.7 | 19.8 | 53.3 |
| | Blood from case of chronic nephritis | 16.1 | 21.4 | 62.3 |
| | Blood from case of chronic nephritis | 20.0 | 17.7 | 54.2 |
| Tunica vaginalis: | Hydrocele fluid | 4.4 | 13.7 | 81.9 |

A microscopic examination of transudates shows only a few leucocytes and some normal endothelium of the pleura or peritoneum, generally in a condition of fatty degeneration.

## EXUDATES

The strictly inflammatory exudates show great variations. We can distinguish macroscopically, serous, chylous, and chyloid, hæmorrhagic, purulent, and putrid. A special variety is the diphtheritic, which is not fluid.

The specific gravity is above 1.018; the reaction is alkaline; the globulin quotient is often high, approaching that of the blood. After long standing there is a deposit at the bottom of the vessel consisting of fibrin, cells, pus, or blood. The fluid may be stained a red color by the solution of the hæmoglobin from the red cells.

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[1] *Joachim:* Wien. klin. Woch., 1902, p. 565.

### SEROUS EXUDATES

In serous exudates a moderate amount of clot separates as a rule from the serum after it is drawn from the body. Microscopically, we find a dense fibrin network containing a few red blood corpuscles, which may be partly or entirely derived from the bleeding at the site of the needle puncture; a few polynuclear leucocytes, lymphocytes, and large mono- or polynuclear cells from the surface of the pleura or peritoneum either single or arranged in masses.

The leucocytes, when stained by the Jenner method, show more or less well-marked neutrophile granulations. Some of the neutrophile leucocytes are mononuclear, but this appearance is usually due to a modification of a normal polynuclear nucleus by which the latter contracts into a mass instead of remaining distributed throughout the cell. This phenomenon is frequently observed in other inflammatory exudates. The leucocytes and the endothelial cells are frequently in an advanced stage of degeneration, depending upon the length of time which the fluid has remained in the chest. Fatty degeneration is common in both types of cells. In case of exudates arising in the pleura without evident cause or after exposure to cold, tubercle bacilli will frequently be found after centrifuging large quantities of the fluid and examining the sediment; or more easily by injecting 10 c.c. of the fluid into the peritoneal cavity of a guinea-pig. A few pneumococci may occasionally be found in serous exudates, especially in cases in which there has been an antecedent pneumonia.

### CHYLOUS EXUDATES

Chylous exudates are seen in cases of rupture of one of the large lymphatic trunks into either the pleural or the peritoneal cavity, or very rarely the pericardium. The fluid in such cases is white and milky and contains a considerable amount of fat, which can be easily demonstrated microscopically by allowing a little of the fluid to dry upon a slide and then staining the fat granules by means of Sudan III or by osmic acid. If the chylous exudate is rendered slightly alkaline with NaOH and then shaken out with ether, the fat will be removed and the fluid become much less opaque, though the opacity rarely clears up entirely owing to traces of albumin which are present.

### CHYLOID EXUDATES

Exudates of this type are seen in carcinoma or sarcoma of the intestines and peritoneum, spleen, pancreas, or ovary, in chronic pleuritis or proliferative peritonitis, in cirrhosis of the liver, and in chronic tuberculosis of the peritoneum. Chyloid exudates have also been occasionally noted in nephritis.

The fluid is not so milky white as that of the chylous exudates, and the amount of fat which can be demonstrated is very small. The opacity is due partly to fat, partly to the fine débris of the broken-down and degenerated cells present in the exudate. In some cases it has been ascribed to nucleo-albumins in suspension in the fluid, while recently it has been shown that the turbidity of many of these chyloid exudates can be explained by the fact that they contain considerable quantities of lecithin.[1]

In order to test for the lecithin, a few cubic centimeters of the fluid should be gently warmed with five parts of strong alcohol. This precipitates the albuminous substances and dissolves the lecithin. If the filtrate is now evaporated down to its original bulk, the lecithin will be precipitated by the removal of the alcohol and the turbidity can be increased by the addition of a little water. Fats will not dissolve in alcohol in this manner, nor produce an emulsion on evaporating the fluid, but collect in oily drops. For the substance, lecithin, there are no simple tests. As it contains phosphorus it is possible to demonstrate that element by fusing the residue obtained by evaporating the alcoholic extract with sodium hydrate and nitrate and testing the aqueous solution of fused product with ammonium molybdate. A yellow precipitate indicates phosphorus.

Wolf[2] has been able to isolate, from a case of chyloid ascites due to carcinoma of the peritoneum, an ester of cholesterin-oleic acid. The fluid did not clear up on the addition of alkali nor on extraction with ether, but when shaken out with chloroform the whole fluid thickened to a paste which in the course of 24 hours again became thin, allowing the emulsified chloroform to settle to the bottom. The ester appears to have united with the euglobulin content of the exudate, as it was with difficulty extracted from the fluid.

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[1] See *Christen* (Cent. f. inn. Med., 1903, p. 181), who holds that the turbidity is due to lecithin.

[2] Beit. z. chem. Phys. u. Path., Bd. v, 1904, p. 208.

A further contribution to the subject has been made by Joachim,[1] who found in a case of cirrhosis with chyloid ascites a fluid which shaking with ether did not clear up. Fractional precipitation with ammonium sulphate resulted in clearing of the fluid in the fraction obtained between one-third and one-half saturation; in other words, the pseudoglobulin portion.

Lecithin could be demonstrated in the precipitate, so that it seems probable that a combination of the pseudoglobulin and lecithin is responsible for the turbidity in some of the cases.

It is evident that more study is needed to clear up the chemistry of the chyloid exudates.[2]

## HÆMORRHAGIC EXUDATES

These occur in persons suffering from hæmorrhagic diathesis, after injuries to the chest, but most often in connection with an active tuberculosis or with new growths of the pleura or peritoneum. A careful microscopic examination of the fluid may therefore frequently give us valuable diagnostic points. Tubercle bacilli may be difficult to find even after concentrating the fluid. A diagnosis is best made by injecting a guinea-pig.

In cases of a new growth of the pleura it may be possible to find small masses of the tumor which have fallen off from the surface of the pleura and are drawn off with the fluid. Such masses are exceedingly difficult to distinguish from similar masses of the pleural endothelium, which also fall off in cases of an acute inflammation of that surface. The cells derived from the new growth are often very large, up to 120 micra. They are characterized, as a rule, by their vacuolation, and generally lie in distinct masses. They are usually in a state of fatty degeneration.

A valuable diagnostic point in the morphology of these cells is the presence of mitotic figures in the nuclei. The examination of such fragments of tumors is best done by embedding and sectioning the preparation in the usual manner, and not by attempting to make a diagnosis with unfixed material. If it is necessary, however, to make a preliminary diagnosis on a fresh specimen, the best stain is a dilute aqueous solution of thionin, followed by the examination of the mass in either water or glycerin.

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[1] Münch. med. Woch., 1903, p. 1915.

[2] For a thorough study of the chemistry of chyloid exudates, see *Bernert:* Arch. f. exp. Path. u. Pharm., Bd. xlix, 1903, p. 32 (Bibliography); *Schulz* u. *Müller:* Deut. Arch. f. klin. Med., Bd. lxxvi, 1903, p. 599; *Mosse:* Int. Beit. z. inn. Med. Fest. v. Leyden, Bd. ii, p. 299.

In hæmorrhagic exudates which have remained for some time in the chest we often find cholesterin crystals. A few of these are also usually found in old serous exudates. They are identified by their rhombic form and notched angles. Occasionally we can find small yellow masses of hæmatoidin, especially in exudates which have remained for a long time in the chest.

## PURULENT EXUDATES

These fluids are usually more or less yellow and thick and deposit a considerable layer of cells on standing. They contain numerous leucocytes, usually of the polynuclear variety, though in gonorrhœal pus eosinophiles are abundant. The leucocytes are frequently in a condition of advanced fatty or albuminous degeneration, and may contain some of the bacteria connected with the infection, notably the gonococcus, micrococcus catarrhalis, and the meningococcus.[1] The nuclei of the leucocytes may be collected in a mass in the center of the cell body and thus simulate myelocytes, though the condition is probably a degenerative one.

In addition to the leucocytes, pus often contains fat and fatty acid crystals, and at times cholesterin. This substance is more abundant in pus which is very old, as, for instance, the degenerated leucocyte débris from a tuberculous abscess.

The morphological examination of fresh pus is far less valuable than the study of a stained smear. Such smears of pus can be made either with a platinum needle or with the edge of a slide, as in smearing blood. They are best stained with the Jenner stain, which not only colors the bacteria sharply, but brings out the granules of the leucocytes. No heat fixation is required with the Jenner stain.

It is necessary, in endeavoring to make a morphological diagnosis of the species of bacterium present in a given specimen of pus, to resort to special stains, and among these the most valuable is that known as Gram's.[1]

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[1] A recent study of the bacteriology of purulent exudates in the pleural cavity by *Bythell*, may be found in the Journal of Pathology and Bacteriology, 1904, vol. ix, p. 359.

**Gram's stain** requires the use of two reagents, aniline water-gentian violet solution[2] and Gram's iodine solution. The first is composed of 5 c.c. of aniline oil, which is shaken up with 125 c.c. of distilled water and the resulting emulsion filtered through a moistened filter paper to remove the excess of the aniline oil which does not pass through the wet paper. To 108 c.c. of this watery solution of aniline oil are added 12 c.c. of a saturated alcoholic solution of gentian violet. The mixture is best used when at least twenty-four hours old, and should be filtered before staining. It keeps for a few days.

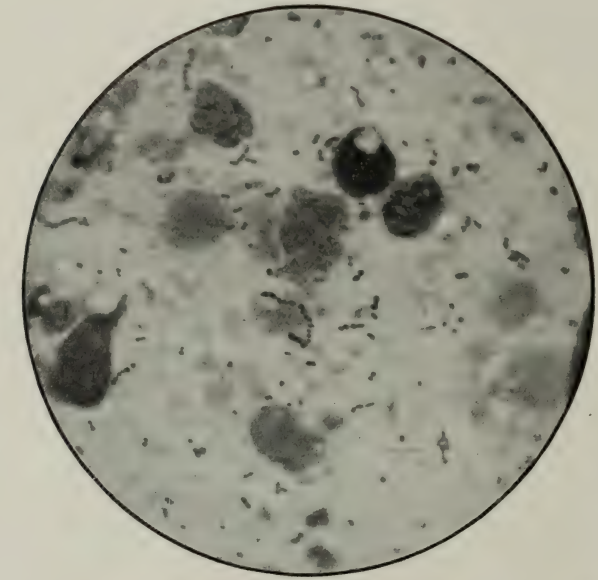

Fig. 171.—Pus from Acute Streptococcus Peritonitis, Showing Streptococci, Leucocytes, and Peritoneal Epithelium. Jenner Stain. Magnified 1,000 diameters.

The Gram's iodine solution contains one part of iodine, two parts of potassium iodide, and three hundred of water.

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[1] *C. Gram:* Fort. d. Med., 1884, p. 185.

[2] *Ehrlich:* Deut. med. Woch., 1882, p. 270.

Smears of the pus are best made on a slide by means of the edge of another slide, as in smearing blood, or by means of a thick straight platinum needle. They are fixed by passing six to eight times through the flame, or until the smear side, which has been away from the flame, is too hot for the hand to bear. The aniline-gentian violet solution is poured on the smear for one to three minutes, and then the excess blotted off with filter paper. It should not be washed off. The iodine solution is then poured on and allowed to act for from one to three minutes. The preparation is then decolorized with strong alcohol for about five minutes, or until all the purple has been extracted from the slide and the smear is only faintly visible as a grayish opacity resembling ground glass. Now, as certain bacteria, and among them the gonococcus, give up their color to the alcohol when treated as above, it becomes necessary to use a counterstain to make them visible. One of the best is a saturated solution of Bismarck brown prepared by adding an excess of the dye to boiling water. After the stain is cool it can be left on the preparation for several minutes, as there is but little risk of overstaining with this dye. The solution keeps only for a few days.

Another counterstain which is easily made is a solution of carbol-fuchsin so diluted as to be quite transparent in layers of an inch in thickness. If used in full strength the carbol-fuchsin would overstain everything and the preparation be valueless. With the dilute solution it is rarely necessary to stain for more than a few seconds to obtain a good result. If the preparation is examined with a low power before putting on the immersion oil and the leucocyte nuclei are found to be stained with the counterstain and not with the gentian violet, the preparation is a suitable one. This does not apply to the thick portions of the smear—they are of no value for study—but merely to the thin portions, where the only satisfactory examination can be made.

**Classification of Bacteria According to Staining Properties.**—Bacteria may be divided into two groups, according to whether they are positive or negative to Gram. By positive we mean that the bacteria retain the primary color or the gentian violet after decolorization with alcohol. The term "negative to Gram" indicates that the bacteria lose the primary color after treatment with alcohol and take up the counterstain, such as Bismarck brown or fuchsin.

| Positive | Negative |
|---|---|
| Tubercle bacillus [1] | Colon bacillus |
| Smegma bacillus [1] | Typhoid bacillus |
| Lepra bacillus | Cholera bacillus |
| Timothy bacillus [1] | Influenza bacillus |
| Anthrax bacillus | Friedländer's bacillus |
| Tetanus bacillus | Plague bacillus |
| Diphtheria bacillus | Diplococcus intracellularis |
| Pneumococcus | Gonococcus |
| Streptococcus | Micrococcus catarrhalis |
| Staphylococcus | Koch-Weeks bacillus |
| Saprophytic cocci of the urethra | Conjunctivitis bacillus of Morax-Axenfeld |

The bacillus of Friedländer, the diphtheria bacillus, and the Diplococcus intracellularis are somewhat variable in their behavior towards Gram's stain, and may or may not decolorize, but the most usual type is taken in the column of the classification table.

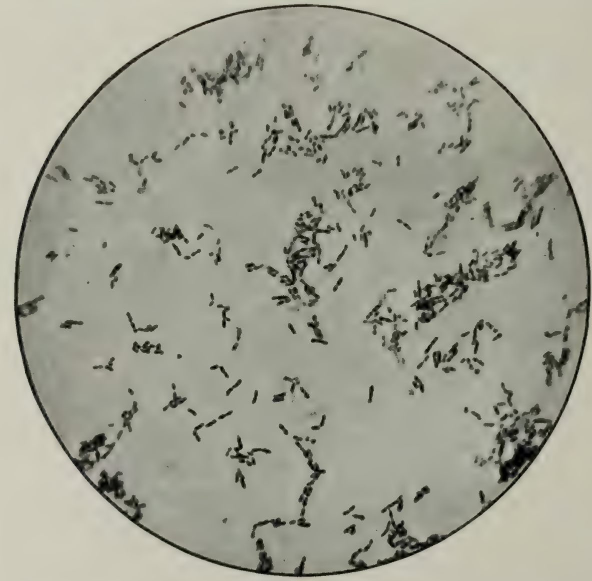

Fig. 172.—Diphtheria Bacilli, Short form. Fifteen Hours' Growth on Loeffler's Blood Serum. Magnified 1,000 diameters.

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[1] These take the Gram stain only when exposed to the action of the aniline gentian violet for twelve to twenty-four hours.

**Gonorrhœal Pus.**—From this table it will be seen that the gonococcus is decolorized or is negative to Gram, while the diplococci of the normal or inflamed urethra are not decolorized or are positive to Gram. In other words, if a preparation is stained as above and small biscuit- or coffee-berry-shaped cocci are seen in the pus cells and are stained with the secondary stain—that is, the Bismarck brown or the carbol-fuchsin—the probability is that they are gonococci, while any of the pus cocci or the cocci which inhabit the urethra will retain the primary stain and therefore will be colored a deep purple. The only exception to this rule is the micrococcus catarrhalis, which, however, is rarely found in the urethra and does not give rise to an acute urethritis clinically, but to a chronic or subacute process which does not resemble the gonorrhœal infection. Absolute distinction between the two forms of organisms can be made only by cultural methods.

**Abscess Pus.**—In the pus from abscesses and other infections a Gram's stain, or even a Jenner, will often permit of a preliminary diagnosis of the species of bacteria present. This diagnosis may be of use in surgical prognosis, and may also be of value in guiding the further study of the material by means of cultures.

An excellent example is the pus from a case of salpingitis, which a morphological examination will usually show to be sterile. The uselessness of abdominal drainage under such conditions is evident. If, however, a large number of streptococci or staphylococci can be shown to be present in the pus, the question of drainage becomes more important.

The pus from the buboes of a case of plague will often show the characteristic short bacilli with marked polar staining.

## CONJUNCTIVAL EXUDATES

The gonococcus is usually found in pure culture in the conjunctival secretion of infants in cases of ophthalmia neonatorum, and the diagnosis is comparatively easy. The rapid determination of the presence or absence of the gonococcus in a conjunctival secretion is of the utmost importance, as perforation of the cornea, with a permanent loss of vision as a result, may take place in twenty-four hours or even less after the infection.

In older children and adults a number of bacteria stand in close causal relation to acute conjunctivitis, among them the streptococcus, the pneumococcus, the diphtheria bacillus, the Koch-

Weeks bacillus, and a bacillus resembling that found in the sputum in influenza.

Another organism which may be present is the diplobacillus, described by Morax and Axenfeld as the determining agent in some forms of both acute and chronic inflammations of the conjunctiva. The organism is a short, thick diplobacillus which is frequently contained in the pus and epithelial cells of the conjunctival discharge, and which is negative to Gram.

The xerosis bacillus is an inhabitant of the conjunctiva in over 50 per cent. of healthy people.[1] This bacillus can not be distinguished by its morphology from the diphtheria bacillus, hence the clinical appearance of the conjunctiva in cases of suspected diphtheria is often more important than the morphological findings. The strepto- or pneumococcus may be mistaken by a careless observer for the gonococcus unless a Gram stain is used. The Koch-Weeks bacillus is in the form of long, slender rods which decolorize by Gram, and which, though they frequently lie in the pus cells, are not liable to be confused with the gonococcus.

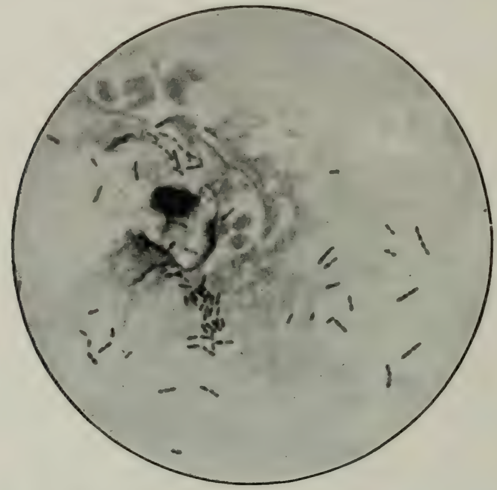

FIG. 173.—MORAX-AXENFELD DIPLOBACILLUS FROM CONJUNCTIVA. (Specimen of Dr. Arnold Knapp.) Magnified 900 diameters.

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[1] See for further details, *Griffith:* The Flora of the Conjunctiva in Health and Disease, Thompson-Yates Lab. Rep., vol. iv, 1901, p. 98. Also *Axenfeld:* Special Bakt. d. Auges, Handbuch d. path. Mikroorganismen, Bd. iii, p. 489.

### DIPHTHERITIC EXUDATES

These exudates occur chiefly on the naso-pharyngeal mucosa and on the tonsils. More rarely they are seen in the trachea and larynx and in the vagina. In a certain proportion of the cases the formation of this exudate is dependent upon the presence of the streptococcus, but more often upon that of the diphtheria bacillus.

In a moderate proportion of the cases of true diphtheria the bacilli may be demonstrated in smears made from the membrane and stained with methylene blue. It is far better, however, to make a preliminary culture upon Loeffler's blood serum and to examine the growth which results.

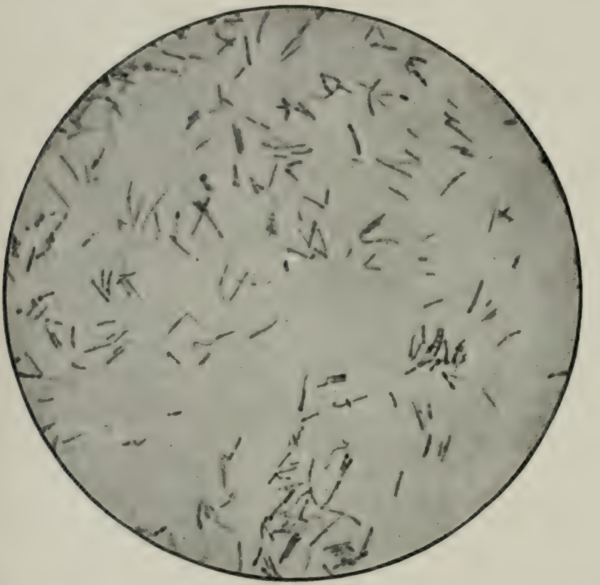

FIG. 174.—DIPHTHERIA BACILLI, LONG FORM. FIFTEEN HOURS' GROWTH ON LOEFFLER'S BLOOD SERUM. Magnified 1,000 diameters.

The technique is as follows: A sterile cotton swab is rubbed over the diseased area—which should not have been treated with antiseptics for twelve hours previous—and then smeared over the slanted surface of the coagulated serum, taking care not to break up the surface of the latter. The tube is then placed in an incubator for from twelve to sixteen hours and a smear made from the whole surface of the growth. The smear is fixed by heat, stained for fifteen to thirty seconds with Loeffler's methylene blue,[1] and examined with an oil immersion lens. If a number of long, slender bacilli with clubbed ends are present, and especially if the staining of the bacillus is very irregular and deeply staining polar granules are present, a probable diagnosis of diphtheria may be made (see Fig. 174).

It will be noted that certain very definite conditions are implied in the positive morphological diagnosis of the diphtheria bacillus.

1. The smear is from a naso-pharyngeal or laryngeal exudate.
2. The growth must be for not much over sixteen hours at incubator temperatures.
3. The bacteria must be grown upon a special medium.
4. The bacilli must show a definite morphology under these conditions.

If the bacilli show the characteristic morphology they may be assumed to be virulent. Tests which have been made by inoculating animals show that in about 98 per cent. of the cases with characteristic morphology the bacteria are virulent.

Special methods of staining have been devised to differentiate the virulent from the non-virulent forms of the diphtheria bacillus, and also to separate the diphtheria bacillus from the xerosis bacillus which is a normal inhabitant of the conjunctival sac. The method devised by Neisser[2] depends upon the demonstration of polar bodies in the virulent diphtheria bacilli. While this stain is useful in many cases, it does not furnish an absolute differentiation between the virulent and avirulent forms, as occasionally bacilli of both types are seen which do not stain characteristically.

**Neisser Method.**—The cultures are preferably grown for from nine to twenty-four hours on Loeffler's solid glucose bouillon-serum medium at a temperature of 34° to 35° C. Smears are made in the ordinary way, dried, and fixed by heat. They are stained for one to three seconds in a solution containing 20 c.c. of concentrated alcoholic methylene blue, 50 c.c. of glacial acetic acid, and 950 c.c. of distilled water. The smear is washed in water and counterstained for three to five seconds with Bismarck brown solution made up by dissolving two grams of Bismarck brown in a liter of boiling water, cooling and filtering. The smear is then washed in water, dried, and examined with an oil immersion lens.

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[1] See Appendix for the composition of this dye, and text-books on bacteriology for the method of making Loeffler's glucose bouillon serum.

[2] Zeit. f. Hyg., Bd. xxiv, 1897, p. 448.

By this method the diphtheria bacilli stain brown with two fairly sharply defined, round or oval, blue granules at either pole. Occasionally a small granule is seen in the middle of the bacillus.

The best means of determining the virulence of a culture is to inject 1 c.c. of a two-day-old broth culture into the subcutaneous tissues of a guinea-pig. The animal will die in one to two days if the growth is virulent. A grayish slough is usually found at the site of the injection and the surrounding tissues are œdematous. The suprarenals are hæmorrhagic.

Mixed infections with the strepto- and the staphylococcus are quite frequent, especially in scarlatina, and a careful search is sometimes necessary to find any diphtheria bacilli in the smear. If bacilli are found of the characteristic morphology, the case should be regarded and treated as a case of true diphtheria.[1]

While the value of throat cultures in diphtheria is very great from the point of view of diagnosis and treatment, it is quite as important to determine the length of time which the diphtheria bacilli remain in the throats of persons who have suffered from the infection, and also to determine the presence or absence of diphtheria bacilli in the throats of those who have been in contact with children suffering from diphtheria. In many cases diphtheria bacilli can be isolated from the throats of healthy nurses who have been working in diphtheria wards.[2] A slight exposure to cold or a reduction of the body resistance by overwork may make these persons liable to an attack of mild diphtheria. The clinical symptoms are often very slight, with practically no rise of temperature and only a slight membrane, often confined to one tonsil. Such cases are usually unwilling to be considered as contagious and to be quarantined; but the writer has found in a number of personally observed cases that the bacilli are fully virulent, and in one case was able to show undoubted infection conveyed by a nurse with bacilli in the throat to a healthy child.

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[1] For further details on the subject of diphtheria bacilli, see an excellent paper by *Beck:* Handbuch der path. Mikroorganismen, Bd. ii, p. 754.

[2] See on this subject a paper by *Graham Smith:* Jour. of Hygiene, vol. iv, 1904, p. 258.

Cases of this type should be regarded as diphtheria, and should be isolated, and repeated cultures should be made from the tonsils and pharynx, until no more diphtheria bacilli can be found.

In children, especially those who have suffered from nasal diphtheria, cultures will reveal the presence of large numbers of characteristic bacilli for weeks and even months after the symptoms of the disease have disappeared. Such children seem to be capable of reinfecting themselves from the nasal sinuses. The writer has seen a number of cases in which the diphtheria bacilli disappeared from the throat after repeated cultures, and the child was again transferred to the general ward of the hospital. A few days or weeks later a free nasal discharge would be noticed, the child's temperature would rise, and finally a membrane would reappear in the throat. Such cases are exceedingly dangerous, as they are capable of transmitting the disease to other children in the same house or ward. It is important, therefore, especially in those cases in which the nose has been involved in the diphtheritic process, to make cultures from the nasal mucosa before considering the child as free from bacteria.

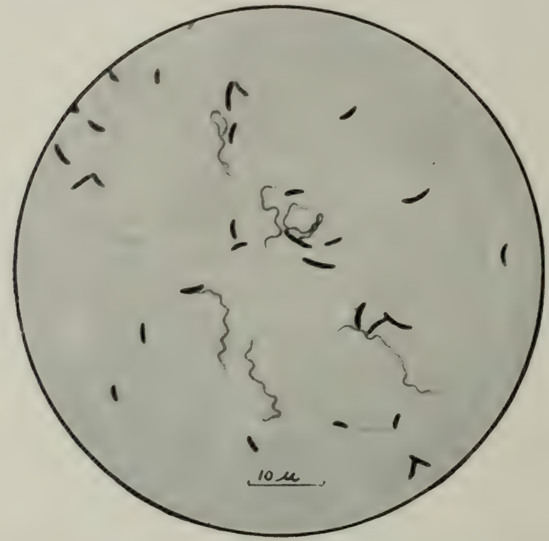

Fig. 175.—Pseudomembranous Angina. Fusiform bacilli and spirochæte. Magnified 1,000 diameters. Line in lower part of cut is ten micra in length.

In uncomplicated cases of pharyngeal diphtheria, the bacilli often persist in full virulence for weeks or months, and it is a serious question for the physician to decide just what shall be done with these children. Painting the throat with strong solutions of silver nitrate will often remove the bacilli temporarily, but reinfection may take place from one of the nasal sinuses. If possible the child should be transferred to the country and be kept out of doors, when the bacteria will rapidly disappear as the child's resistance increases.

In connection with the length of time for which bacilli may persist in the throat, some interesting statistics have been collected.

In 345 cases of diphtheria out of 654 examined [1] the bacilli could not be isolated from the throat after the disappearance of the membrane. In a few cases they could not be found a short time before the final healing of the local process. Of the remaining 309 cases 211 showed bacilli for ten to twenty days after the membrane had disappeared; 51 for twenty to thirty days; 41 for thirty to sixty days; 4 for sixty to ninety days, and 2 for ninety to one hundred and twenty days.

The bacteria persisted longer in mild cases. Some of the cases showed bacilli after two or three negative cultures had been seen. This is probably due to reinfection of the pharynx from the nasal sinuses or the crypts of the tonsils after the surface of the pharynx had become free of diphtheria bacilli. The bacilli under these conditions are virulent in a large proportion of the cases, and are capable of setting up an acute diphtheria in healthy persons.

**Pseudomembranous Angina** (*Vincent's Angina*).[2]—A somewhat rare type of diphtheritic inflammation of the tonsils and pharynx is occasionally met with in which two forms of parasites may be isolated, one a fusiform bacillus, the other a spirochæte. The same organisms are also found in ulcerative stomatitis.

The clinical course of the disease is very chronic and is characterized by an extensive membrane of a dirty greenish color which may be confined to the tonsils or cover the tonsils and pharynx. The throat in these cases resembles very much that of ulcerative secondary syphilis.

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[1] *Prip:* Zeit. f. Hygiene, Bd. xxxvi, 1901, p. 283.

[2] *Plaut:* Deut. med. Woch., 1894, p. 920; *Vincent:* Ann. de l'Inst. Pasteur, tome xiii, 1899, p. 609; *Mayer:* Am. Jour. Med. Sci., vol. cxxiii, 1902, p. 187; *Fisher:* ibid., vol. cxxvi, 1903, p. 438.

The bacillus is a slender fusiform rod from 6 to 12 micra long, pointed at each end, and somewhat suggestive of the diphtheria bacillus. It usually decolorizes by Gram, and is not motile.

The spirochæte is sufficiently described by its name. The number of convolutions varies from 3 to 11. (See Fig. 175.) It is actively motile and negative to the Gram procedure, and occasionally it appears as a short crescent.

The fusiform bacillus has been cultivated, the cultures having a marked fetor. It is doubtful whether the spirochæte has been isolated in pure culture. The importance of the recognition of these two parasites and the conditions which they incite is that, as a rule, the cases are considered as diphtheria or syphilis from the clinical appearance of the membrane. It has been found that the diphtheria bacillus may be present in the membranes in conjunction with the other organisms, rendering a morphological diagnosis impossible. The presence of the Spirochæte pallida will differentiate the syphilitic ulcerations.

### PUTRID EXUDATES

Putrid exudates are found in both the pleural and peritoneal cavities from the entrance of pus from the gangrenous areas near these cavities or from the perforation of ulcers of the stomach and intestine, and also in connection with malignant tumors. The puncture fluid is often of very offensive odor, especially in abscesses to which the colon bacillus has easy access, as, for instance, an abscess following an acute appendicitis.

Microscopically, degenerated leucocytes, cholesterin, fatty acid needles and hæmatoidin are generally found. Besides the colon bacillus, a large number of other bacilli may be frequently identified in the puncture fluid, but, as a rule, cultural results are necessary before an accurate determination can be made of the bacteria present.[1] Abscesses in the substance of the liver are characterized, as a rule, by the absence of bacteria, by the advanced degeneration which the leucocytes and liver cells show, and by the presence of bilirubin and cholesterin crystals as well as crystals of leucin and tyrosin, and occasionally by the presence of the amœba dysenteriæ.

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[1] An important paper on the bacteriology of putrid empyemas is that by *Guillemot, Hallé* et *Rist:* Arch. de méd. exp., tome xvi, 1904, p. 571. (Bibliography.)

## II. CYST FLUIDS

**Echinococcus Cysts.**—The fluid obtained by puncturing an echinococcus cyst is, in uncomplicated cases, perfectly clear, free from albumin, and contains a characteristically large amount of sodium chloride, which can easily be identified by allowing a drop of the fluid to crystallize out on a slide and examining for the square crystals of the salt. The specific gravity varies between 1.008 and 1.013. Very rarely, no trace of any morphological elements can be found. Occasionally only a few hæmosiderin or cholesterin crystals and a few fatty cells are present. Usually, however—and this is the most important point—we find, in such fluid, remnants of the scolices of the echinococcus. When these are absent, a careful search of the fluid will frequently reveal the presence of small hooklets derived from the scolices. These hooklets furnish an absolute diagnosis of the presence of the echinococcus embryos. (See Figs. 101 and 103, pages 360 and 361.) Occasionally, the cyst may have become infected and the contents may be bloody or purulent, in which case the finding of the hooklets is rendered more difficult.

**Parovarian Cysts.**—The contents of parovarian cysts are usually clear and limpid or slightly opalescent. Occasionally a faint yellow tint is present, and still more rarely the cyst may contain chocolate-colored blood and masses of hæmatin. The specific gravity is from 1.002 to 1.010. Pseudomucin is not present. Albumin may be found in traces or not at all, except when bleeding has taken place into the lumen of the cyst.

**Ovarian Cysts.**—The cyst contents obtained by puncturing an ovarian cyst of the multilocular type is usually quite thick and stringy, and shows a very variable specific gravity, which may lie between 1.005 and 1.050. The physical properties are sufficient for a diagnosis. The fluid contains usually a considerable amount of albumin and also pseudomucin, which is precipitated neither by acetic acid nor by boiling, but is precipitated by alcohol and is distinguished from mucin by these reactions. The albumin must be removed before this reaction can be carried out.

If some of the alcoholic precipitate is heated with dilute hydrochloric acid, a substance is split off from the pseudomucin which reduces Fehling's solution. This reducing action is due to glucosamin. As glycogen may also be present in the alcoholic precipitate, some of the substance should be dissolved in water and digested at 37° C. with some saliva. If reduction is obtained the remainder of the pseudomucin must be similarly treated to break up the glycogen, the mixture reprecipitated and the purified pseudomucin treated with 2 per cent. hydrochloric acid.

The cyst contents are usually of a yellow color, but may be dark red or even of a chocolate brown. Microscopically, we find red and white blood cells, masses of blood pigment, cholesterin crystals, fatty cells, and cells containing large vacuoles. An especially valuable diagnostic point is the finding of cylindrical or beaker cells filled with mucus.

**Hydronephrosis.**—The contents of the hydronephrotic sac is usually clear, more rarely reddish or dirty yellow and somewhat cloudy, and shows a specific gravity of 1.010 to 1.015. Urea and uric acid are also present and only a small amount of albumin. The presence of the urea and uric acid is only of moderate value from a diagnostic point of view, because these two substances may also be found in the contents of ovarian or pancreatic cysts, and they may be lacking in an old hydronephrotic fluid. The microscopic findings are, as a rule, practically negative.

**Pancreatic Cysts.**—The puncture fluid which is obtained from pancreatic cysts varies greatly in its physical properties, depending upon the anatomical nature of the cyst and the length of time the exudate has remained in the cyst cavity. The exudates which have been rapidly formed, either in traumatic cysts or in cysts connected with malignant new growths of the pancreas, are usually hæmorrhagic and have a high specific gravity—that is, from 1.020 to 1.030. The color may be due to methæmoglobin, and occasionally the cyst contains granular masses of hæmatin produced by the action of the digestive fluids excreted by the pancreas. Cholesterin is occasionally present and serum albumin. Ferments are present in the cyst contents and may be used to identify the fluid when the exudation has existed but a short time. The contents of old cysts rarely give evidence of the presence of either the proteolytic or fat-splitting ferments. The presence of the diastatic ferment is of comparatively little importance, because such ferments are present in the fluid of other types of cysts. Only a marked action on starch is significant of pancreatic cyst fluid. If the fluid from a suspected cyst digests egg albumin disks or fibrin, or, as suggested by Boas,[1] acts upon the proteids of milk, the presence of pancreatic secretion in the cyst contents may be considered probable. For the milk test mix the fluid with *fresh* milk, heat to 37° C. for several hours, precipitate the casein with acetic acid, and test the fluid by the biuret reaction. The test should always be controlled by a parallel one using milk without cyst fluid. Fat splitting ferments may be tested for by the method given on page 577.

**Cammidge Reaction.**[2]—The urine is freed from albumin by boiling, and rendered acid with hydrochloric acid. Forty c.c. of the clear filtrate is mixed with 2 c.c. of hydrochloric acid (sp. gr. 1.16) and the mixture is boiled on a sand bath in a flask having a long-stemmed funnel in the neck to act as a condenser. After boiling for a few minutes the flask is cooled in a stream of water, the contents are made up to 40 c.c., and the excess of acid is neutralized by adding 8 grams of lead carbonate. After standing for a few minutes, the flask being cooled in water, the contents are filtered through paper until a clear filtrate is secured. At this stage any glucose which may be present may be removed by fermentation. This filtrate is shaken with 8 grams of powdered tribasic lead acetate, and the solution is filtered until clear. The excess of lead is removed either with a stream of hydrogen sulphide, or by shaking the filtrate with 4 grams of finely powdered sodium sulphate, the mixture being raised to the boiling point and then cooled quickly in a stream of cold water. The lead sulphate should then be removed by filtration. Ten c.c. of the final filtrate are then made up to 17 c.c. with distilled water and added to 0.8 grams of phenylhydrazin hydrochloride, 2 grams of sodium acetate, and 1 c.c. of 50 per cent. acetic acid. The mixture is boiled on a sand bath for ten minutes in a small flask. The hot solution is filtered through a small filter, already moistened with hot water, into a test tube, and made up to 15 c.c. In well marked cases of pancreatic inflammation a light yellow flocculent precipitate should form in a few hours; but in chronic cases the preparation should be left over night. Under the microscope the flocculi consist of long, light yellow, flexible, hairlike crystals, arranged in sheaves, which on being irrigated with 33 per cent. sulphuric acid melt and disappear in from ten to fifteen seconds after the acid touches them.

The clinical value of this reaction is still undecided. It is certainly not pathognomonic of any pancreatic lesion, though most often found in interstitial pancreatitis. In diabetes only 75 per cent. of cases give the reaction. It is frequently seen in gallstone obstruction of the common duct and may be present in cirrhosis of the liver and in some of the cases of acute hemorrhagic pancreatitis. It has been noted in general peritonitis without lesions of the pancreas. A moderate proportion of the cases of tumors of the pancreas give the reaction, which, therefore, does not differentiate between pancreatitis and malignant disease. It may appear after taking gum arabic or after excessive smoking (Porter). The administration to the patient of considerable doses of calcium salts interferes with the test. Experimentally the reaction has been obtained after induction of acute lesions in or crushing the pancreas, liver, or spleen, and after implantation of pancreatic tissue in the abdomen.

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[1] Deut. med. Woch., 1890, p. 1095.

[2] *Robson* and *Cammidge:* The Pancreas, Philadelphia, 1907.

## III. CYTODIAGNOSIS[1]

The study of the types and the proportions of the leucocytes in the fluids which may be obtained from the pleural, peritoneal, and cerebro-spinal cavities has been designated cytodiagnosis by Widal, who, with his pupils, has contributed most extensively to the subject. The diagnostic value of the results obtained were at first considered to be of great importance, but recently a number of exceptions have been discovered to what were at first laid down as rules, and while the method seems likely to take its place as a useful adjuvant to other means of studying the exudates, it can not in any way replace them.

**Obtaining the Fluid.**—The fluids are obtained either in the course of aspiration for therapeutic purposes, or, more frequently, in the withdrawal of small quantities of fluid for diagnosis.

The syringe should be one which can be easily sterilized, and preferably made wholly of glass. It is of advantage to have needles made of an alloy of platinum and iridium, as they can be easily sterilized by heating to redness in a flame.

For exploratory purposes it is not necessary to sterilize the syringe other than by drawing into it some 1 to 20 carbolic acid and then removing this substance as far as possible by sterile salt solution or sterile water, such as can be obtained from any hot-water tap. If, however, the fluid is to be used for bacteriological examination also, the sterilization of the syringe should be carried out either by heating to 150° C. or by boiling for fifteen minutes in a 1 per cent. sodium carbonate solution.

The skin over the region of the puncture should be carefully cleaned with green soap and water, followed by ether and a 1 to 1,000 solution of bichloride.

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[1] The literature of this subject has been collected by *Brion:* Cent. f. allg. Path., Bd. xiv, 1903, p. 609. See also *Labbé:* Cytodiagnostic, Actualités Médicales, Paris, 1903; *Ravaut:* Cytodiagnostic, Paris, Naud, 1901. An excellent paper on the cytology of pleural fluids with reference to etiology and diagnosis, is that by *Musgrave:* Boston Med. and Surg. Journal, vol. cli, 1904, p. 317.

When the skin is exceedingly tough it may be necessary to make a small incision with a pointed scalpel, having previously rendered the area to be incised anæsthetic by means of a spray of ethyl chloride. The needle is then plunged through the intercostal space or through the abdominal wall or into the spinal canal between the third and fourth lumbar vertebræ. For spinal puncture a needle from 5 to 10 centimeters in length is necessary, and the caliber should be considerably greater than that of the ordinary hypodermic needle, for it is difficult to obtain sufficient quantities of a thick fluid through a fine needle. There is also the additional risk of a sudden movement of the patient breaking the needle and leaving the tip in the tissues.

The fluid when obtained should be promptly centrifuged before the formation of any clot. If it is impossible to examine it promptly, the clot should be broken up by shaking the fluid in a small flask which contains a few glass beads. Small Erlenmeyer flasks should be sterilized at 150° C. and kept ready for use. It has been found that the proportion of leucocytes obtained by prompt centrifugization, and also after defibrinization, is practically the same, though Widal and Rivaut have noted that there may be a slight diminution in the relative number of polynuclears, which adhere to the fibrinous flocculi and are thus removed.

It is necessary to centrifuge the fluid because of the small number of cells which are usually present, the concentration of the exudate rendering the examination much more rapid and satisfactory. This is especially true of sero-fibrinous pleuritic and peritoneal exudates, and also of the cerebro-spinal fluid, as but few elements are usually present in such exudates. If possible from 15 to 20 c.c. should be withdrawn, though fair results can be obtained from 1 to 2 c.c.

The fluid should always be examined within twenty-four hours, as otherwise changes take place in the morphology of the leucocytes which render recognition exceedingly difficult.[1] All fluids for examination should be kept on ice to avoid bacterial growths.

The use of a high-speed electrical centrifuge permits the obtaining of a considerable amount of deposit in about five minutes. A drop of the deposit in the tip of the centrifuge tube is removed by a pipette with a long fine tip, and the drop spread on a slide and allowed to dry in the air. It may be stained and fixed either by the Jenner or Wright stain, or fixation may be carried out separately with strong methyl alcohol for five minutes, and then the preparation stained with methylene azure and eosin as for blood.

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[1] *Patella:* Deut. med. Woch., 1902, p. 16.

The Jenner preparations are often not very satisfactory, for the strongly alkaline serum takes a deep blue with the methylene blue of the dye, and this can not be easily removed by washing without decolorization of the granules of the leucocytes. The preparations are clearer if the sediment in the centrifuge tube is shaken up with 0.85 per cent. saline solution and again thrown down. This washing removes most of the serum. The smears may also be fixed by heat either at 115° C. for ten minutes, or by raising the slide rapidly to 140° C. as recommended by Rubinstein, and stained with the Ehrlich triacid dye.

## CYTOLOGY OF NORMAL FLUIDS

The observer should, if possible, become familiar with the cells normally found in the pericardial and pleural fluids obtained from healthy persons or at autopsy after death by accident. Under such conditions the cells are very scanty, but as a rule a considerable number of red and white corpuscles are present, the white cells being proportionately much more numerous than in the circulating blood. A few endothelial cells are also likely to be found. Large quantities of red corpuscles are usually derived from an injury of some small vessel during the course of the puncture, and the subsequent filling of the needle with a small amount of blood. The proportion between the leucocytes in normal fluids is not the same as in the blood, but no constant rules can be made. Polynuclears, neutrophiles, eosinophiles and lymphocytes are all present; but they are often so greatly altered by maceration in the serum that they are difficult to recognize. Usually, however, eosinophiles are very much more abundant than in the blood.

## CYTOLOGY OF PATHOLOGICAL FLUIDS

### PLEURAL EXUDATES

1. *Primary Tuberculous Pleurisy.*—These exudates are characterized by an excess of polynuclear cells during the first few days

of the inflammation, but as it is rarely possible to obtain the exudate so early, this neutrophilia has been usually overlooked.

In the later stages of a purely tuberculous infection the lymphocytes form the major portion of the white cells. They are often so altered that it is difficult to distinguish the cell body, but it is usually possible to separate them easily from the polynuclear neutrophiles, and especially from the eosinophiles, the granules of the latter being very resistant. The number of red cells found is usually small. Endothelial cells with large nuclei and a basophilic cell body are present in very moderate numbers.

2. *Secondary Tuberculous Pleurisy.*—In case the exudate in pleurisy is secondary to a pulmonary tuberculosis or has developed after a hydropneumothorax, the morphology of the cells in the exudate is very considerably altered. The lymphocytes, and especially the polynuclears, show signs of degeneration of the nuclei often with very marked fragmentation, and free nuclei are also found. The endothelial cells are single and not collected in masses. At times the polynuclears can be identified only by their granules, but occasionally even these disappear. The neutrophiles are often more numerous than the lymphocytes. In one case of a pleurisy arising in a woman, a subject of pulmonary tuberculosis, Widal and Ravaut found 54 per cent. of eosinophiles, 6 per cent. of neutrophiles, and 40 per cent. of lymphocytes. The difference in these two forms of tuberculosis is probably due to a secondary infection by the bacteria of the lung.

### ASEPTIC PLEURISIES

1. *Transudates of Heart and Kidney Disease.*—The transudates of heart disease and nephritis are characterized by the presence of large numbers of endothelial cells grouped in masses usually containing from six to ten cells, which can be recognized by their large nuclei. The cell body is often indistinct. The protoplasm frequently takes a pink stain with eosin. In a recent hydrothorax these masses of cells often cover the entire field of the microscope. In the later stages some lymphocytes appear and the masses of endothelial cells show evidences of degeneration. The cell bodies are often swollen or fatty or may be dissolved in the fluid. Pulmonary abscesses, infarcts, or emboli modify the nature of the transudate by the addition of a considerable

number of red cells and polynuclears which may rise even to 95 per cent. of the leucocytes present.

2. *Exudates Following New Growths of the Pleura or Peritoneum.*—The cytological formula of these exudates is not characteristic, but in a few cases, masses of the new growth may be obtained in the puncture fluid. These are often difficult to distinguish from the large endothelial cells of the pleura, but occasionally mitotic figures can be seen in the cell nuclei rendering diagnosis of a new growth very probable. The peritoneal exudates which follow from the new growths are not characteristic, and tumor cells are only rarely found.

3. *Exudates During the Course of Leukæmia.*—The leucocytes found in these exudates correspond with those found in the blood. If the exudate is due to an infection, polynuclears are usually the most abundant. When the exudate is due to mechanical causes, eosinophile, neutrophile and basophile myelocytes have been seen together with a certain number of large endothelial cells. In one case of Sicard and Monod,[1] the proportions were those of the circulating blood. In one case of ascites arising in a case of myelogenous leukæmia, Milchner found a very large proportion of basophiles, more than were present in the blood. Labbé,[2] in a case of lymphatic leukæmia, found a very large number of red cells with numerous lymphocytes, identical with those found in the blood, but no polynuclears nor masses of endothelial cells.

4. *Syphilitic Pleurisies.*—Widal and Ravaut, in a case of pleurisy arising during the secondary period of a syphilitic infection, found a few large endothelial cells, 22 per cent. of lymphocytes, 37 per cent. of eosinophilic myelocytes, and 6 per cent. of neutrophilic myelocytes.

5. Exudates occurring in connection with abscess of the liver, rheumatism, and diphtheria have not shown any facts of interest.

### SEPTIC PLEURISIES

1. *Pneumococcus Pleurisies.*—These pleurisies are characterized by the presence of polynuclear neutrophiles in large numbers, usually somewhat proportionate to the richness of the bacterial infection. In the early stages a few endothelial cells may be

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[1] Soc. méd. des Hôpitaux, 1900.

[2] *Labbé:* Le Cytodiagnostic, 1903, p. 53.

found, but later the lytic action of the serum alters the morphology of the leucocytes very greatly. In the later stages of cases which are improving, the polynuclears may be replaced by lymphocytes. If the pleurisy, on the other hand, becomes a purulent one, the polynuclears increase in number, the nuclei become fragmented, and the granules are often very difficult to demonstrate.

2. *Streptococcus Pleurisies.*—Streptococcus pleurisies show a large number of polynuclears and a few isolated endothelial cells. The fluid usually becomes rapidly purulent in type, with an enormous increase in the neutrophiles.

3. *Typhoid Pleurisies.*—Typhoid pleurisies show a considerable number of red cells and excess of lymphocytes, and usually a number of large masses of endothelial cells. In one case, Widal and Ravaut found an increase in the eosinophiles to 23 per cent., associated with 66 per cent. of lymphocytes and 10 per cent. of mononuclears and endothelial cells.

## DIAGNOSTIC VALUE OF CYTOSCOPY

The results of the cytoscopic examination of pleural fluids may be of very considerable diagnostic value in determining the nature of the process inciting the exudation. The method is comparatively simple and rapid, and can be carried out by the practitioner with materials always at hand. It must be remembered, however, that it is frequently difficult to make out the exact type of the cells which are present. The exudate is not always isotonic with the body fluids, and as a rule the morphology of the cells is considerably altered by lytic processes. It is especially difficult to distinguish between polynuclear neutrophile leucocytes with pycnotic nuclei and softened cell bodies and those of the lymphocyte group.

Animal inoculations and cultural procedures are to be preferred in all cases before a final diagnosis, but as these are complicated and time-consuming methods, the practitioner will often find that a cytological study of pleural fluids will give him results of very considerable diagnostic value.

## PERITONEAL EXUDATES

A study of the cells contained in peritoneal exudates does not furnish us with many conclusions of diagnostic value. Occasionally in general carcinosis of the peritoneal surface, masses of large cells may be found in the aspirated fluid, sometimes containing mitotic figures in their nuclei.

Tuberculous inflammations of the peritoneum may show either an excess of lymphocytes or an excess of polynuclears. In acute peritonitis the polynuclears are exceedingly abundant. The results so far obtained from the study of the cells of the exudates from the pericardium, the tunica vaginalis, and the joints are of but little importance. Bacteriological examination of the fluids is likely to furnish facts of much greater value.

## IV. INOSCOPY

A method termed inoscopy,[1] which has been used in demonstrating tubercle bacilli, depends upon the digestion of the fibrinous network formed in the serous or cerebro-spinal fluids by the action of pepsin. The clotting of the fluid extracts the bacteria by entangling them in the fibrin meshwork. The fibrinous mass is freed as much as possible from the supernatant fluid, and then treated with about 30 c.c. of a digestive mixture, composed of

| | |
|---|---|
| Pepsin | 2 grams |
| Glycerin | 10 c.c. |
| Hydrochloric acid (specific gravity, 1.18) | 10 " |
| Sodium fluoride | 3 grams |
| Distilled water | 1,000 c.c. |

The mixture is placed in an incubator for twenty-four hours, the presence of the sodium fluoride preventing bacterial growth. As soon as the fluid has become homogeneous it is to be thoroughly centrifuged and smears made from the deposit.

The smears are mixed with a small quantity of egg albumin in order to fix the bacilli on the slide, and after passing nine times through the flame, are stained in the usual manner for the demonstration of tubercle bacilli. The quantity of fluid used must be very considerable to demonstrate bacilli in peritoneal or pleural exudates. The fibrinous coagulum from four or five liters of fluid should be employed, if possible. With cerebro-spinal or puncture fluids the amount of fluid which can be used is necessarily much less, but the bacilli can be demonstrated in more than half the cases of tuberculous meningitis.

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[1] *Jousset:* Semaine Médicale, 1903, p. 22.

The presence of tubercle bacilli in the blood of patients suffering from miliary tuberculosis has been demonstrated by allowing 30 or 40 c.c. of this fluid to flow into about 200 c.c. of sterile water. Small fibrinous flocculi form in this mixture in a short time.

Some observers have not obtained as many positive results as Jousset, and attention has been called to the possibility of mistaking small crystals of blood pigment for tubercle bacilli. It must also be remembered that tubercle bacilli are somewhat altered in their morphology by the digestion with an artificial gastric juice, and do not take as deep a stain with the carbol-fuchsin as is usually obtained.

The method is especially useful where a rapid diagnosis is necessary. Otherwise animal inoculations are preferable.

## V. LUMBAR PUNCTURE

In puncturing the spinal canal in order to obtain cerebrospinal fluid for diagnostic purposes, one may use either a stout needle with a stylet, the former being connected by means of a short rubber tube to a glass tube, or an aspirating syringe with a needle attached. The patient should lie on the left side near the edge of the bed with the knees drawn up as near as possible to the chin. The needle is entered slightly to one side of the median line between the third and fourth lumbar vertebræ. As soon as the dural sac is reached the fluid enters the canula. The glass tube can be attached with its rubber connection, and the height to which the fluid rises may be determined on a graduated scale. As soon as this is noted the glass tube can be tipped over so that the fluid runs out into a suitable sterile receptacle. The needle used for the purpose of lumbar puncture should be from 4 to 10 centimeters long, and with a lumen from 1 to 1½ millimeters. The shorter and smaller needles are for children. The larger needles should be supplied with a stylet, because it is easy to block the end of the needle with small tissue fragments or to obtain a considerable quantity of blood in the lumen, which interferes with further diagnostic procedures. The stylet is removed when the needle passes the dura. The length of the rubber tube is 20 to

40 centimeters, and that of the glass tube is 10 to 15 centimeters, with a diameter of about 2 millimeters.

The horizontal position is preferred for puncturing, though some recommend a sitting posture with the arms of the patient resting over the back of the chair. In this position, however, the pressure of the spinal fluid is difficult to measure, and sudden falls of pressure in the spinal canal are likely to set up dangerous circulatory changes.

Careful disinfection of the skin is essential as a preliminary to the operation, and the instruments should have been thoroughly sterilized by boiling in 1 per cent. sodium carbonate solution.

General anæsthesia is usually not necessary, but the pain of the skin puncture may be mitigated by freezing with ethyl chloride. Passing the needle through the deep muscles of the back is practically painless; the stretching of the periosteum and the puncturing of the dura is somewhat painful but not seriously so. After removal of the needle the point of puncture should be covered by a sterile bandage or a little collodion.

The patient should be kept quiet in bed for at least twenty-four hours, in order that equal pressure may be established in the cerebro-spinal cavity and no unpleasant symptoms result from the procedure.

Despite its apparent simplicity a lumbar puncture is not without danger to the life of the patient. Giarrè[1] noted unpleasant symptoms in one case out of thirty-four punctures. This was in a child two and one-half years old, who suffered from hydrocephalus. While the fluid was being drawn off rather rapidly he became unconscious, but no further ill effects followed.

Gumprecht[2] reports two deaths following lumbar puncture in children. In both cases the symptoms pointed to brain tumor, and indicated an increase in the intracerebral pressure. Death resulted from respiratory paralysis. In another case the needle was broken off by a sudden movement of the patient, but without any serious consequences. A severe pain in the back and partial paralysis were observed, but both symptoms disappeared spontaneously.

The fluid from a lumbar puncture in cases of disease may give a pressure of 200–800 mm. of water, normal being 60–100 mm.

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[1] Revista critica di Clinica Medica, Anno I, 1900, p. 105.

[2] Deut. med. Woch., 1900, p. 386.

Usually from 20 to 100 c.c. can be obtained. The reaction is alkaline to litmus. It contains a trace of proteid, usually globulin, and a small quantity of a reducing substance which in some cases has been shown to be pyrocatechin, in others glucose. Cholin, cholesterin, and a large amount of potassium as compared to sodium salts have been noted. The specific gravity is about 1.005 to 1.010, and the fluid freezes at – .60° to – .65° C. In acute inflammations of the meninges the freezing point approaches that of the blood, i. e., – .56° C.

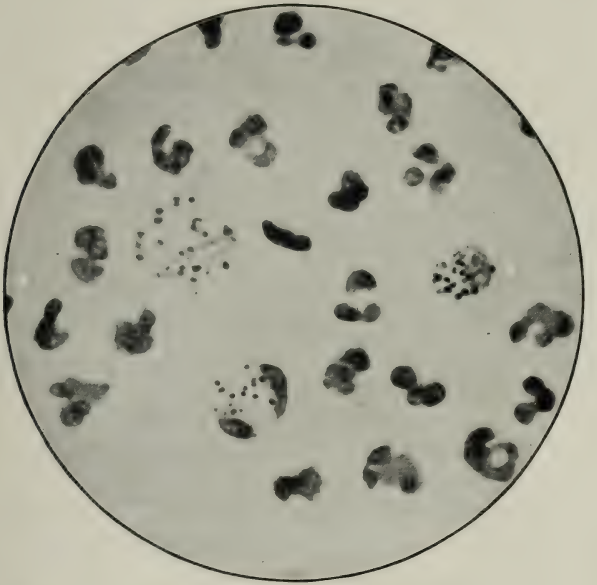

FIG. 176.—MENINGOCOCCUS FROM SPINAL FLUID OF A CASE OF ACUTE CEREBRO-SPINAL MENINGITIS. Magnified 1,000 diameters.

1. In tuberculous cerebro-spinal meningitis the fluid is usually clear, rarely slightly opalescent. Very frequently a delicate fibrinous clot separates in which the tubercle bacilli are especially abundant. The best procedure is to obtain the fluid in a sterilized conical tube and to centrifuge it immediately and examine the deposit for bacilli. A positive result is obtained in about 50 to 75 per cent. of cases. The fluid usually contains a considerable number of leucocytes and a few red cells. The fluid from a case of tuberculous meningitis in the early stage may

show an excess of polynuclear neutrophiles. Later in the course of the disease the lymphocytes increase in number and soon are present in a great excess. Occasionally polynuclears are difficult to find in the fluid from meningitis of long standing. Very rarely exceptional cases have been seen, in which, notwithstanding the tuberculous nature of the disease and its chronic course, the polynuclears are more abundant than the lymphocytes.

2. In acute forms of meningitis the fluid may be quite transparent, especially in those cases due to the meningococcus. More often it is somewhat opalescent, or it may contain blood; it is rarely thick and purulent. The last condition is more common in streptococcus meningitis. The meningococcus is identified by its intracellular position and by the fact that it is negative to Gram. It may be differentiated from the gonococcus by its growth on ordinary media.

The pneumococcus is also a frequent etiological factor in the production of an acute meningitis, and may be identified with reasonable certainty by its form and the presence of capsules. In rare cases the cause of the disease may be the typhoid bacillus, which may be recognized by its agglutination under the influence of serum from a typhoid patient.

Cytoscopic examination shows an excess of polynuclear neutrophiles.

3. In chlorosis, which is accompanied by severe headaches, the pressure of the cerebro-spinal fluid is frequently quite high, and these headaches have been cured by removal of the fluid. The fluid shows no especial characteristics.

4. In brain tumors the clear fluid contains only a few leucocytes and a trace of albumin. Sometimes cases are observed in which the albumin is quite abundant, and occasionally an increased amount of sugar may be found, or at least a substance which reduces Fehling's solution.

5. The fluid which can be obtained after fracture of the skull or after an apoplectic attack often contains a considerable amount of blood.

## VI. ANIMAL INOCULATION

The inoculation of a susceptible animal and demonstration of characteristic lesions is one of the most certain means of verifying the presence of small numbers of tubercle bacilli in a fluid which contains very few of these organisms. In a similar manner the presence of the pneumococcus and the anthrax bacillus may be rapidly determined by inoculating a white mouse with a little of the suspected fluid and demonstrating the organism in stained smears of blood. Other species may be isolated in the same way, but the practitioner is likely to confine his investigations to the demonstration of the tubercle bacillus.

The guinea-pig is the most suitable animal for this purpose, and young, rather small animals are the most susceptible. The material to be tested is to be injected either into the peritoneal cavity or subcutaneously, according to the nature of the infection. If, for instance, the presence or absence of tubercle bacilli in serum aspirated from the pleural cavity is to be established, the material should be injected into the peritoneal cavity. The number of bacilli is likely to be small, thus necessitating the injection of at least 10 c.c. of the fluid, and it is difficult to inject this amount subcutaneously and have all the fluid retained. If, on the other hand, the substance to be tested is purulent in character, or is likely to be contaminated with other germs than the tubercle bacillus, it is best to inject in the groin, for then, if an acute infection with suppuration occurs, the wound is superficial and easily heals after a short time, while the tubercle bacilli, if present, go on to cause their characteristic lesions in the lymph nodes and other organs of the body. If contaminated fluid must be injected into the peritoneal cavity, as is sometimes the case with urine from a suspected case of genito-urinary tuberculosis, a number of animals should be injected, so that if some die from general peritonitis, others may escape and later develop tuberculous lesions.

The post-mortem examination of the animals should be conducted as follows:

The body is to be fastened on a soft pine board either by nails driven through the four extremities or by the use of steel hat-pins or the small glass-headed push-pins sold by dealers in photographic supplies for pinning out flexible films. The fur of the animal should be soaked with a 2 per cent. lysol solution to prevent the hair from flying about, and in order to kill any parasites which may be on the animal.

If cultures are to be made, the skin should be incised in the middle line from the sternum to the pubes with sterile forceps and scissors after a thorough burning of the line of incision. If, however, merely an anatomical inspection of the tubercles and a morphological examination of their contents are to be made, nonsterile instruments may be used.

The abdominal cavity should be opened, the ribs cut through on either side, the intestines removed by cutting off the mesentery close to its insertion on the spinal column, and careful search should be made for small pearly gray tubercles or yellowish gray masses which are usually cheesy lymph nodes. One of the small miliary tubercles may be crushed between two slides, and after drying and fixing by heat, the smear may be stained with carbolfuchsin, decolorized by acid and alcohol, counterstained with methylene blue, and examined with an oil immersion for the characteristic red rods of the tubercle bacillus. The cheesy nodes should be incised and a portion of the contents scraped out, spread on a slide, and fixed and stained as above.

After an intraperitoneal injection the peritoneum and the omentum will be found to be covered with larger and smaller tubercles, and the various organs, especially the liver and spleen, are usually abundantly filled with large miliary tubercles. (See Fig. 177.)

Cultures should be made from the nodes if an absolute diagnosis is necessary for scientific purposes, but usually the demonstration of acid-resisting bacilli is sufficient. As a rule, not very many bacteria will be found in the cheesy material.

When testing milk or butter, it is necessary to inoculate a second animal with some of the tubercles, as the milk or grass bacilli often present are capable of forming pseudo-tubercles when directly injected into the peritoneal cavity; but if a second inoculation be made, no such tubercles will be formed in the case of the Moeller's bacillus, while the tubercle bacillus will form characteristic tubercles.

When the fluid to be tested is injected under the loose skin of the inner surface of the thigh, the course of the lesion is less rapid than when the material is placed directly in the peritoneum. The animals usually lose weight slowly and show a gradual rise in temperature after the first week or so. The site of the injection usually suppurates and remains as a granulating area for some time. The inguinal lymph nodes on the same side as the injection enlarge during the third week and can be palpated. The infection gradually spreads up the lumbar and retroperitoneal nodes, and

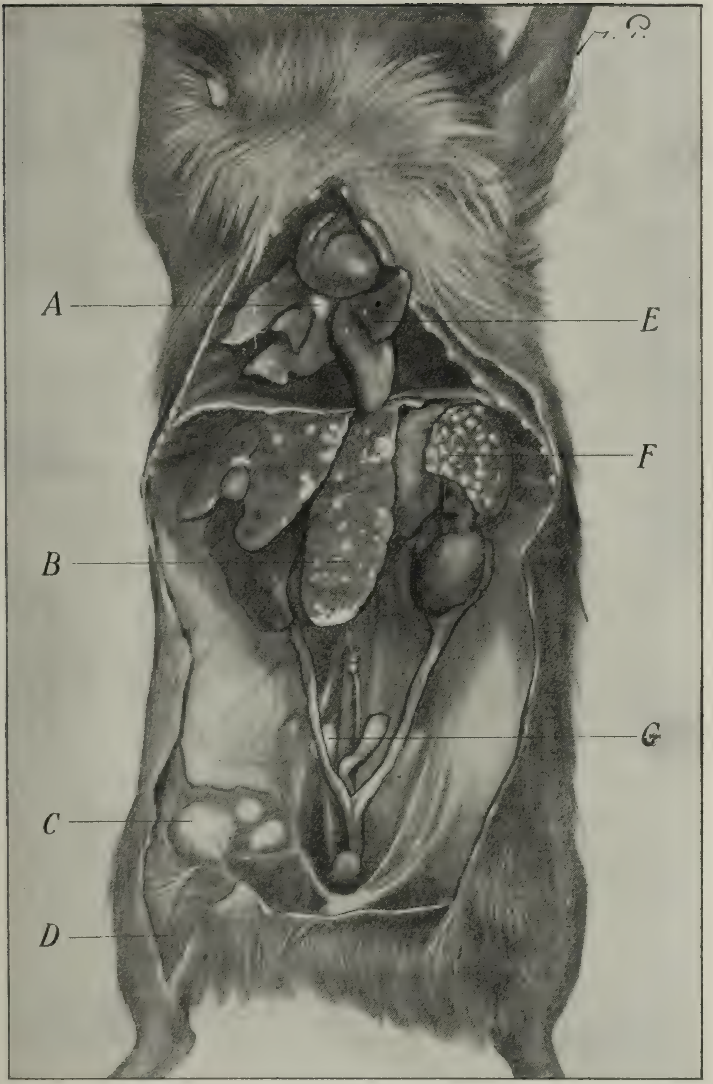

Fig. 177.—Dissection of Guinea-pig, Six Weeks After Inoculation in the Right Groin with Tuberculous Material.

*A*, cheesy bronchial lymph node ; *B*, tubercles in liver ; *C*, large, cheesy lymph node of the inguinal group ; *D*, site of inoculation ; *E*, small miliary tubercles in lung ; *F*, spleen showing miliary tubercles ; *G*, tuberculous lymph node of the lumbar group.

passing under the diaphragm, involves the bronchial nodes and the lungs during the fourth and fifth weeks. The animal often dies at the end of the sixth or seventh week with a general tuberculosis of the nodes and viscera.

This method of inoculation is to be preferred to the intraperitoneal one in all cases where the specimen is contaminated or when a thick deposit can be obtained from the suspected material, or when solid tissues are to be tested, as, for example, in examining lymph nodes for tuberculosis in cases of apparent Hodgkin's disease. The solid material can be placed in a little pocket on the inner surface of the thigh, made by incising the skin with a pair of scissors after lifting up a fold with the forceps. A pocket is then formed by pushing the scissors or a platinum spear under the skin, separating it for a centimeter from the areolar subcutaneous connective tissue. The solid material or pasty sediment is then transferred to the pocket by a platinum spatula.

## VII. TUBERCULIN REACTIONS

In addition to the subcutaneous use of tuberculin as a means of diagnosticating tuberculous lesions, as mentioned on page 615, three new methods have recently been introduced and widely studied, though without reaching final conclusions as to their value at the time of this writing (1909). These reactions are the cutaneous reaction devised by von Pirquet, the ophthalmo reaction, described practically simultaneously by Wolff-Eisner [1] and Calmette [2], and that obtained by the use of tuberculin salve as suggested by Moro.

**Cutaneous Tuberculin Reactions.**—The method of von Pirquet is as follows: The inner side of the forearm is washed with ether, and two drops of Koch's old tuberculin are placed on the cleansed area at a distance of about 8 cm. from each other. The skin covered by the drop is then gently scarified. The tuberculin is allowed to remain in position for ten minutes; the clothing is then replaced, no bandages being necessary. A slight swelling is usually produced immediately after the scarification, which in the course of a few hours is surrounded by a delicate reddish border. Most of these symptoms disappear in twenty-four hours;

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[1] Die Ophthalmo- und Kutan-Diagnose der Tuberkulose, 1908.

[2] *Petit:* Le diagnostic de la tuberculose par l'ophtalmo-réaction, 1907.

they are entirely due to the irritation of the skin by the trauma. If the reaction is positive, the scarification area begins to swell in about twenty-four hours, though occasionally, especially in children, no changes are seen for forty-eight hours. The diameter of the reddish papule so produced is from 10 to 15 mm. The center may be pale and show some serous blisters, usually there is no pus formed.

In about forty-eight hours the reddening begins to disappear and in four or five days there is no palpable alteration of the skin. A slight pigmentation and scaling usually follow the positive reaction. Usually there is no temperature and the process causes no general disturbance.

A repetition of the inoculation after several days usually gives rise to a positive result and may excite previously inoculated areas. This reaction is not indicative of tuberculosis, but is a so-called hypersensibility reaction.

Moro [1] has suggested the use of a salve made of equal parts of old tuberculin and anhydrous lanolin. A small fragment of this salve, about the size of a pea, is rubbed into the skin of the thorax or abdomen. In tuberculous cases, after about twenty-four to forty-eight hours, small pale nodules of a diameter of 1 to 2 mm. appear on the spot inoculated. If the reaction is not a strong one, these nodules disappear in the course of a few days. A more severe reaction usually results in the formation of reddish miliary nodules at the end of twenty-four hours. The reaction remains limited to the place of inunction and is usually slightly itchy. Very strong reactions may give rise to a large number of reddish nodules with extension of the inflammation beyond the area of inunction. There may be some exudation from the inflamed surface. This strong reaction is rarely seen.

**The Ophthalmo Reaction.**—One to two drops of a solution of old tuberculin diluted 1 to 100 in physiological saline solution are placed in the conjunctival sac, near the inner canthus. The lids are kept apart for a few moments in order that absorption of the tuberculin may take place. Only one eye should be treated. A positive reaction may assume four grades and usually begins in from six to eighteen hours. There may be: (1) Mere reddening of the caruncle and the inner surface of the lower lid. (2) The reddening may extend to the bulbar conjunctiva. (3) There may be a suppurative conjunctivitis with marked injection of the palpebral conjunctiva. (4) There may be a hæmorrhagic condition of the palpebral and bulbar conjunctivæ with profuse secretion of fibrino-purulent exudate. In the severe cases there may be a rise in temperature. Occasionally very severe reactions have been seen leading to partial or complete destruction of the eye.

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[1] Münch. med. Woch., 1908, p. 216.

The most frequent complication is a phlyctenular ulceration which may leave permanent opacities after the inflammation has subsided. Many of the serious reactions have been due to a second application of tuberculin to an eye already tested. This almost invariably results in a very severe reaction, which is not indicative of tuberculosis but is due to what is known as hypersensibility or anaphylaxis. Subcutaneous injections of tuberculin should never be made after trying the ophthalmo reaction, for the conjunctiva remains hypersensitive for some time after such treatment, and the subcutaneous injection of tuberculin may give rise to a very active conjunctivitis, even when the eye appears perfectly normal.

Sharp criticism has been made in regard to the ophthalmo reaction because of numerous reports of very serious injury to the eye following the application of tuberculin to the conjunctival sac. Approximately 1 or 2 per cent. of cases treated give very severe reactions causing some discomfort and often requiring local treatment for relief of pain and reduction of the inflammatory process. Some fifty cases of severe injury to the eye are now on record. In some a keratitis has been produced, in others ulceration of the cornea with or without perforation. In a few cases the reaction has been so severe as to cause total loss of vision, and, more rarely, destruction of the eye by the extension of the inflammation to the interior of that organ. These severe reactions are chiefly seen in persons with a fairly active tuberculosis, and in all probability the eye is already the seat of some local tuberculous process which is lit up by the application of the tuberculin. These considerations render the general use of the ophthalmo reaction in the future somewhat questionable, as it is never possible to judge whether the reaction will be a severe or a mild one, and as no method at present exists for the standardization of tuberculin in a manner comparable to the control of diphtheria antitoxins.

Definite contraindications to the use of tuberculin in the conjunctival sac are (1) any inflammation of the eye or conjunctiva; (2) previous treatment of the eye with tuberculin; (3) recent subcutaneous injections of tuberculin.

It is as yet much too early to judge of the practical value of these methods in diagnosticating tuberculosis. The general opinion is that the Moro reaction is preferable for use with adults and the von Pirquet reaction with children. Many thousands of cases in which the eye test has been employed have been published, with the result that about 95 per cent. of cases of evident tuberculosis gave a positive reaction. About the same percentage of cases react to the cutaneous inoculation of von Pirquet. The results with tuberculin salve are somewhat less frequent, though the number of cases reported is still relatively small. As a rule persons with extensive and active tuberculosis do not give the reactions. Some of those with quiescent lesions give the reaction; others do not. A considerable proportion of healthy persons, varying in statistical collections from 10 to 25 per cent., give a positive reaction though there is no clinical reason for suspecting tuberculosis. Some of these reactions in healthy persons are no doubt due to an incautious use of tuberculin by repeating the application in the same eye. Such a procedure usually gives rise on the second application to a severe conjunctivitis, but is, as previously stated, a phenomenon of hypersensibility and not an evidence of tuberculosis. The reactions correspond roughly with those obtained by the subcutaneous injection of tuberculin, but are much less unpleasant in their application and results. The fact that the reaction may be given by a patient who has two lesions, one, a quiescent tuberculosis, the other, for example, an acute simple bronchitis, may lead to a false diagnosis of acute pulmonary tuberculosis.

## VIII. OPSONIC INDEX

That the polynuclear neutrophile leucocytes of the blood have the power of taking up inert particles, bacteria, and protozoa, has long been known. It has been shown by Denys and Leclef (1895), and more recently by Wright (1903) that the phagocytosis of bacteria by leucocytes is rendered more active by the presence in the blood of a body or bodies which have been termed by Wright "opsonins." The substance in the serum which exerts this action does so in virtue of changes produced in the bacteria and not by its influence on the leucocytes. The virulence of the organism modifies to some extent the amount of phagocytosis, extremely virulent strains being less subject to the engulfing action of the leucocytes.

It is probable that the opsonins represent a somewhat specific group among the antibodies present in the blood, and while they are influenced in their action by the agglutinins, they are not the same as these substances, nor are they identical with the aggressins, or bacteriolytic or precipitating substances. The opsonins are present in normal blood, but are increased during the reaction of the body to infectious agents, such as bacteria and trypanosomata. Wright has based upon these observations a theory of immunity in which he claims that the rise and fall of the opsonic power of the blood is an index of the rise and fall in the resistance of the body to the action of the pathogenic agent, and that, therefore, if the amount of the opsonic power can be determined, we have a practical guide in diagnosis and a means of regulating and controlling the therapeutic use of certain forms of active immunization.

## TECHNIQUE

For the determination of the opsonic power of the blood it is necessary to have a bacterial emulsion, normal human blood serum, serum from the person to be tested, and a suspension of fresh leucocytes.

1. *Bacterial Suspension.*—The organism inciting the infectious disease must be isolated and determined. Cultures of the same organism or of a similar organism must then be made for use in the tests. In case staphylococci, streptococci, pneumococci, gonococci, or colon bacilli are found, agar slant cultures are allowed to grow for from six to twenty-four hours, and the bacteria are then washed off the surface of the slant with 0.85 per cent. sodium chloride solution. The emulsion is allowed to stand for a short time, and the supernatant turbid fluid is pipetted off and centrifugalized in a small tube for five minutes. The slightly opalescent supernatant fluid is then removed in a pipette and used as the bacterial suspension.

When tubercle bacilli are employed in obtaining the index, the bacilli should be heated to 100° C. for ten minutes on three successive days, and then ground up in an agate mortar or a watch glass with 1.5 per cent. salt solution. The mixture should then be centrifugalized for five minutes and the supernatant fluid, which should be slightly opalescent, pipetted off and used in this form to mix with the serum and leucocytes.

In order to get the suspension of bacteria perfectly even, Wright suggests emulsifying it by drawing up the fluid, after centrifugalization and just before making the mixture of the serum and leucocytes, into a long pipette (Fig. 178, No. I) whose tip has been carefully broken off square. The tip of the pipette containing the bacterial suspension is rested against the bottom of a watch glass and the fluid is squirted in and out of the pipette. The rapid flow of fluid through the small chinks between the flat end of the pipette and the watch glass breaks up any clumps of bacteria and makes a homogeneous mixture (Fig. 178, No. IV).

2. *Phagocyting Leucocytes.*—The washed leucocytes are obtained by allowing about twenty to thirty drops of blood from a healthy person to fall into a centrifuge tube already nearly filled with a solution containing 1 per cent. sodium citrate and 0.85 per cent. sodium chloride. The citrate in this mixture prevents coagulation and the corpuscles are separated from the fluid by centrifugalization, and washed twice by suspending in 0.85 per cent. solution to remove all traces of serum and citrate. After the final washing, the upper grayish layer of corpuscles, which contains most of the leucocytes, is pipetted off and forms the suspension of "washed corpuscles," the leucocytes of which form the phagocyting agent.

3. *Preparation of Serum.*—The finger of the patient whose index is to be obtained is punctured and about 0.5 c.c. of blood is collected in a small curved tube (Fig. 178, No. V). The blood serum in this tube is separated from the corpuscles either by the centrifuge or by spontaneous retraction of the clot. A stock of normal serum is similarly obtained from four or five healthy individuals, and after the serum has separated equal quantities of each specimen are mixed with the others, thus making a "pooled" or average serum free from individual variations, which is considered by Wright to form a normal or a standard for the determination of the opsonic power of the blood.

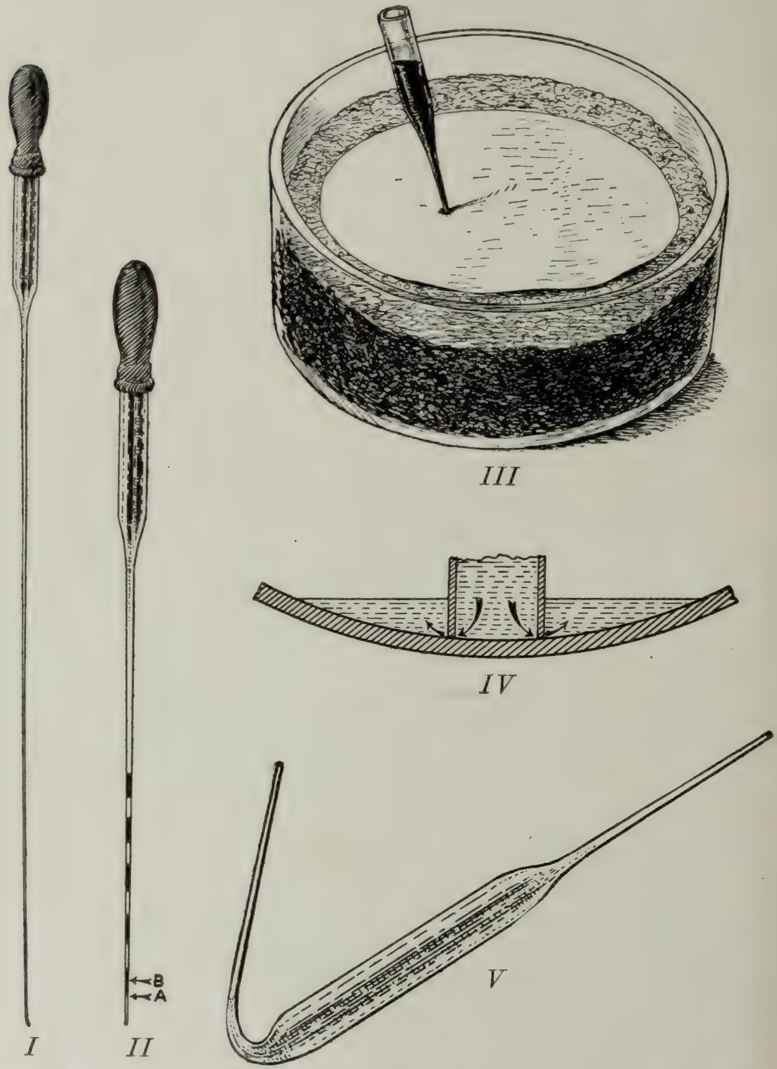

FIG. 178.—APPARATUS FOR DETERMINING OPSONIC INDEX. *I*, Pipette for handling serum; *II*, pipette with measured columns of serum separated by air spaces; *III*, dish with moist sand for holding bacterial mixtures or blood serum; *IV*, diagram of currents in watch glass for emulsifying bacteria; *V*, Wright capsule for collecting blood.

4. *Preparing the Opsonic Mixture.*—A capillary pipette (Fig. 178, No. II) is made by drawing out a glass tube about 6 mm. in diameter to a fine capillary not over 1 mm. in caliber. An ink or wax pencil mark is made on the stem of this pipette about 2 cm. from the capillary end. The pooled serum is then drawn up into the capillary to the mark; by relaxing the pressure on a bulb an air bubble is allowed to enter the column, and then an equal volume of the suspension of white corpuscles is allowed to rise in the tube to the mark. Another air bubble is admitted, and, finally, the bacterial emulsion is also drawn up. The mixture is then blown out into a watch glass or on to a slide, and rendered homogeneous by alternately sucking the fluid into the capillary and blowing it out again. After this has been done four or five times the mixture is drawn into the pipette again, the capillary end is sealed, and the whole is placed in an incubator for fifteen minutes. An exactly similar preparation is made of the serum whose opsonic power is to be tested. At the end of fifteen minutes, the pipettes are removed from the incubator, the ends are cut off with a file, the corpuscle-serum-bacterial-emulsion-mixture is blown out on a glass slide which has either been previously heated in a flame or slightly roughened with very fine emery paper (No. 00) and the drop is smeared with the edge of a cover glass or another slide. The smear is allowed to dry; it is then stained with Jenner (p. 82), or fixed in methyl alcohol and stained with carbol thionin (p. 88).

For tubercle bacilli it is necessary to stain with carbol fuchsin. The slide is fixed in alcohol or a saturated solution of mercuric chloride for ten minutes. It is then washed off, heated for ten minutes in the dye, decolorized with 2 per cent. sulphuric acid, washed in alcohol, and lightly counterstained with methylene blue.

The smears are then examined with an oil immersion lens and the number of bacteria found in a hundred leucocytes is counted. The same is done for the serum whose index is to be determined. The average number of bacteria per leucocyte is the phagocytic index of each serum. The ratio between the phagocytic indices is the opsonic index.

C. E. Simon[1] prefers to obtain the percentage of phagocyting to non-phagocyting leucocytes seen on examining the smear, rather than to determine the number of bacteria in the individual leucocyte. By comparing the number thus obtained to a corresponding number in the pooled normal serum an index is obtained which is comparable to that of Wright. As the percentage of phagocyting cells is to a certain extent dependent upon the organisms present, it is an advantage to work with an emulsion of bacteria which in normal blood serum does not give a higher percentage of phagocyting leucocytes than fifty. This will allow for an increase in the index of the patient's blood to nearly two. Simon also suggests that these determinations be controlled by corresponding examinations of blood in dilutions of 1–20, and 1–40, as occasionally large amounts of opsonin may be shown to be present by the powerful action of the diluted serum as compared to the normal.

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[1] Journal of Exp. Medicine, vol. viii, 1906, p. 651.

**Clinical Applications.**—The first use of the results obtained by the determination of the opsonic index was in the treatment of tuberculosis with Koch's tuberculin. Wright showed that persons suffering from tuberculosis usually have a low or fluctuating index to tubercle bacilli, that large inoculations of tuberculin lower the index (negative phase) for a considerable time, and that small inoculations lower the index temporarily and then cause a rise above normal (positive phase). According to Wright's theories, a permanent negative phase shows that the patient is not resisting the tuberculous process; whereas a high index shows that the patient is making a good fight against infection. The results of Wright's investigations have not as yet proved the necessity of employing the index in the administration of tuberculin, the patient's general condition being still considered as a sufficient guide by many of experience. But it is unquestionable that the results of the opsonic method have shown that large doses of tuberculin are often unnecessary, if not dangerous.

The opsonic index is also used in treating other infectious diseases, especially those forms in which the bacterial infection is localized or chronic. The best results obtained so far have been in chronic furunculosis, in chronic sinus infections, in the sycoses, and in other localized skin infections. In many reported cases the inoculation of the patient with considerable quantities of pure cultures of killed bacteria of the same species as that giving rise to the disease resulted in remarkable improvement. Favorable results have been obtained in gonorrhœal arthritis, in colon bacillus pyelitis and cystitis, and in chronic wound infections, especially those following mastoiditis. In these cases the index to the specific organism is usually found to be low, and suitable injections raise the index against the organism to a high point. In general, vaccine treatment may be tried in any infection which is well localized, as shown by the absence of fever and other evidences of systemic reaction; in other words, when the lesion is so walled off by granulation tissue that the infective agent does not come in free contact with the body fluids and induce the formation of immune bodies. While it is generally assumed that the administration of vaccines in cases of generalized infections is not only useless but dangerous to the patient, Wright thinks that under certain circumstances it may be justifiable. This conclusion is based upon successfully treated cases, and upon the opinion that possibly the effect of injecting bacteria into the tissues from which their products may be slowly diffused in the body may be to incite a reaction, even when the same organisms are exerting grave effects on the patient while circulating in the blood. The question, however, is still open, and many unsuccessful attempts at such treatment have been recorded, far outnumbering the successful ones.

The opsonic index has also been used diagnostically, for example, to differentiate between a tuberculosis of the kidney and a new growth of that organ. If the index to tubercle bacilli is extremely low, the disease is probably tuberculosis; if it is higher than normal, it is unlikely to be tuberculosis. In the same fashion, if a case of general infection shows a very low index to the infecting organism, the prognosis is bad; if high, the reverse.

Difficulties in the application of the procedure are many. The methods of obtaining the opsonic index are extremely tedious and time-consuming, and if the results are to be accurate within 15 to 20 per cent., a very considerable experience is necessary. Even those who have worked for several months under Wright's directions not infrequently show variations of 50 to 100 per cent. in obtaining the index of the same patient. It is doubtful if the actual opsonic power of the serum can be determined by this method, and even if it were known it is far from certain that it would be an index of the degree of resistance against the disease.

Certainly there is no such parallel between the amount of bacterio-agglutinins or lysins and the patient's general condition.

The procedure is much less generally used than it was three or four years ago, and even the Wright school now considers it unnecessary in following vaccine treatment with staphylococci. On the other hand, vaccine treatment, without the aid of the opsonic index, is becoming more widely used, and in chronic localized infections its value is generally admitted.

The determination of the opsonic index will probably in the future be left to special workers.

**Preparation of Vaccines.**—In the case of the gonococcus or tubercle bacillus it is necessary to use stock vaccines made from strains which have been accustomed to growth on artificial media. Such preparations are now sold commercially. With organisms which are easily cultivated it is better to prepare an "autogenous" vaccine of organisms obtained from the lesion to be treated. This is especially important in using colon bacillus, streptococcus and pneumococcus, different strains of which vary so widely, and better results are obtained with autogenous staphylococcus suspensions. In case of a mixed infection the organisms concerned should be isolated and grown separately, as in mixed cultures one is almost certain to overgrow the rest. In preparing a vaccine all glassware and solutions should be sterile.

The preparation of a vaccine comprises three steps: first, the cultivation of the organism in sufficient quantity; second, the determination of the strength of the culture; and, third, the sterilization of the organisms by submitting them to a temperature which will kill them without destroying their antigenic properties. The technique for isolating and cultivating the organisms can be found in any textbook of bacteriology.

The bacteria are grown by spreading them with a needle well over the surface of six to ten slant agar tubes or, better, plates. The latter are conveniently inoculated by mixing the original culture with sterile broth and transferring five or six drops of the broth to each plate with a sterile capillary pipette. The plant is spread by tilting the plate from side to side. For all cocci an agar containing 4 or 5 per cent. peptone gives a more abundant growth. For pneumococcus and streptococcus it is often necessary to add 0.5 c.c. of ascitic fluid to each plate, or, better, to use plates of Loeffler's blood serum. Some workers use gelatin-agar media, but these are too soft to be very satisfactory. The growth is then incubated for twenty-four hours in working with staphylococci; forty-eight hours with pneumococci or streptococci; and twelve to eighteen hours with B. coli. About 2 c.c. of salt solution are then added to each growth and the bacteria scraped from the surface with a sterile platinum needle. In using plates, the plate is tilted to one side and the needle bent in the shape of a hockey stick. Unless the suspension is to be filtered, great care is necessary to avoid cutting the surface of the medium and taking up particles of agar with the emulsion. The mixture is then transferred to a sterile test tube by means of a capillary pipette. The coarser particles are allowed to settle to the bottom and the opaque supernatant fluid transferred to another tube containing a few 2 mm. glass beads. The upper part of the tube is drawn out in the blast lamp, broken off, and sealed (or plugged with a sterile rubber stopper), and the tube shaken vigorously for 15 to 60 minutes, preferably in a machine. Much trouble is avoided if the suspension is first filtered through a small sterile filter paper moistened with salt solution.

The suspension is standardized, according to Wright, by counting the number of bacteria against the cells of normal blood. Take a pipette like that used in opsonin work with a mark about 1 cm. from the tip. Take up blood from a finger prick to the mark, admit a bubble of air, take up bacteria to the mark and then about 3 units of salt solution. The fluid is then blown out on a slide and mixed thoroughly by drawing in and out of the pipette. A small drop is transferred to another slide and a thin even smear made by drawing the end of a slide across this. The smear is stained with Jenner. Before counting a paper disk with a central hole about 3 mm. square is placed in the ocular of the microscope by resting it on the diaphragm, in order to constrict the field. The number of bacteria and red cells in a number of contiguous fields is then counted until 200 red cells are recorded. As there are 5 million red cells in a cubic millimeter of blood the number of bacteria in a cubic centimeter of the suspension may be easily estimated by multiplying the number of bacteria seen in counting 200 red cells by 25,000,000. A short trial will convince any one of the inaccuracy of this method, but it is quite accurate enough for clinical purposes. In fact, some workers simply use the opacity of the emulsion as a guide.

The suspension is then sterilized by heating in a double water bath for half an hour at as low a temperature as possible; 56° C. is sufficient for most pneumococci, and will kill some staphylococci, but other strains require 60° and some 62°. Streptococci require 60° to 65°; colon bacilli, 60°. A plant is then made on agar and incubated for twenty-four hours to test its sterility. If there is growth the vaccine must be heated at 2° higher temperature and again tested for sterility. (Wright advises incubating the vaccine before testing.) Before use the stock vaccine is diluted with salt solution containing 0.25 per cent. lysol so that 1 c.c. contains from 1.5 to 5 times the desired dose. Vaccines should always be kept in vessels containing beads so that an even suspension can be made before injection.

If many vaccines are made up it is simpler to prepare a few small centrifuge tubes for standardizing. A strong test tube of 1.25 cm. diameter is melted 3 cm. from the open end and drawn out until the inside diameter is about 3 mm. The narrow portion is filed, broken off, and sealed so as to leave about 1 cm. This forms a tube with a fine funnel shaped tip in which small sediments can be measured. It is graduated by measuring 0.02 c.c. of mercury into it with a carefully graduated pipette (such as used with the Sahli hæmometer), shaking the mercury to the bottom, and marking the upper border with a diamond pencil. The mark should be about 0.5 cm. from the tip. If the bore near the tip is fairly uniform, fractions and multiples of this unit can be estimated with sufficient accuracy; or if desired the tube can be further graduated with the proper amount of mercury. In centrifugalizing, the tubes are floated in ordinary metal holders about two-thirds filled with glycerine.

These tubes are filled two-thirds with the filtered bacterial suspension and centrifugalized until the compact sediment is up to the mark or above. The fluid and bacteria above the mark are removed with a capillary pipette. One c.c. of salt solution is added and mixed with the sediment by means of the pipette and transferred to a tube containing beads. This gives a 2 per cent. stock suspension which contains about 5,000,000,000 staphylococci or 2,000,000,000 colon bacilli to the cubic centimeter. A capillary pipette is used to clean the tubes after use.

*Administration and Dosage.*—This really belongs to therapeutics, but the following general rules may be given. Injections are made subcutaneously either in the deltoid region or, if large in volume, in the abdominal wall. They are given at intervals of 4 to 10 days in gradually increasing doses. Staphylococci are begun at 50,000,000, and increased to 2,000,000,000 or even 5,000,000,000 (in infants 10,000,000 to 1,000,000,000). Streptococci and pneumococci are begun at 3,000,000 or 5,000,000 and increased to 100,000,000. With colon bacilli also the initial dose should be small, 5,000,000 to 10,000,000, but may usually be increased to 2,000,000,000 or above. In gonorrhœal arthritis Cole uses 300,000,000 to 1,200,000,000. There is usually a little redness and tenderness about the site of inoculation and often a slight rise in temperature with malaise, but any marked systemic reaction indicates that the dose is too large. The practice of following the opsonic index during treatment has been generally abandoned. In typhoid immunization Wright advises two injections of 1,000,000,000 and 2,000,000,000 at an interval of from ten days to two weeks.[1]

The following schedule may be followed in the treatment of staphylococcus infections. Injections are given five days apart.

Stock vaccine, 2 c.c. of 2 per cent. suspension (5,000 million per c.c.).
Dilution No. 1, 0.05 c.c. stock + 0.95 c.c. 0.25 per cent. lysol in salt sol.
“ “ 2, 0.3 “ “ + 1.2 “ 0.25 “ “ “ “ “
“ “ 3, 1.35 “ “ + 0.15 “ 2.5 “ “ “ “ “

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 1st | dose, | 3 | minims | of | dilution | No. | 1 = | 50 | million | cocci. |
| 2d | “ | 6 | “ | “ | “ | “ | 1 = | 100 | “ | “ |
| 3d | “ | 3 | “ | “ | “ | “ | 2 = | 200 | “ | “ |
| 4th | “ | 5 | “ | “ | “ | “ | 2 = | 330 | “ | “ |
| 5th | “ | 8 | “ | “ | “ | “ | 2 = | 500 | “ | “ |
| 6th | “ | 2 | “ | “ | “ | “ | 3 = | 600 | “ | “ |
| 7th | “ | 3 | “ | “ | “ | “ | 3 = | 900 | “ | “ |
| 8th | “ | 4 | “ | “ | “ | “ | 3 = | 1,200 | “ | “ |
| 9th | “ | 5 | “ | “ | “ | “ | 3 = | 1,500 | “ | “ |
| 10th | “ | 7 | “ | “ | “ | “ | 3 = | 2,100 | “ | “ |

Larger doses are usually unnecessary.

## IX. RABIES

In the nervous system of animals and human beings suffering from rabies, especially in the large ganglion cells of the hippocampus major, small oval or round bodies with one or more nuclei are constantly present (Fig. 179). These bodies were originally described by Negri[1], and have since been studied by a number of observers. In size they average from 10 to 15 micra. In animals killed with "fixed virus," obtained by repeated passages through rabbits, the bodies are very small measuring only 1 to 3 micra in diameter. Occasionally in animals suffering from "street rabies" very large forms are seen, measuring from 23 to 27 micra in length and 6.5 micra in width.

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[1] *Wright:* Antityphoid Inoculation, Westminster, 1904.

The general opinion at present is that these bodies are of protozoal origin and are probably the cause of rabies. Whether or not this opinion is confirmed by future researches, it has been found that the demonstration of these bodies in the cells of the brain is equivalent to a positive diagnosis of hydrophobia. They have been found in nearly 100 per cent. of unselected cases

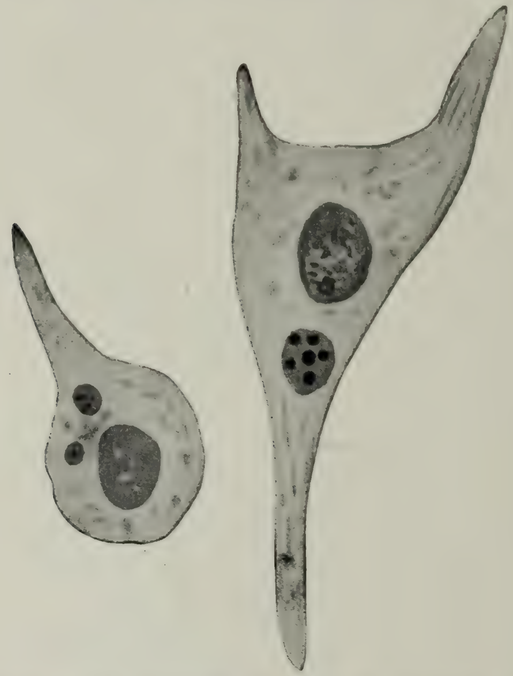

FIG. 179.—NERVE CELLS CONTAINING NEGRI BODIES. Magnified 2,000 diameters.

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[1] Zeitschrift f. Hygiene, Bd. xliii, 1903, p. 507. For further studies and a review of the bibliography, see *Williams* and *Lowden:* Journal of Infectious Diseases, vol. iii, 1906, p. 452.

of "street rabies" in dogs, when the brain cells from the hippocampus major were examined by suitable technique. Control examinations of a large number of brains from animals or human beings dying from other diseases than rabies have not shown the presence of these peculiar bodies.

The technique for rapid diagnosis is as follows[1]: A small fragment of the gray substance, about 3 mm. in diameter, is placed on a slide and covered with a cover glass. The cover glass is then gently pressed down on the brain tissue, crushing it on the slide. Then the cover glass is gently drawn along the slide, by which procedure the nerve cells are left flattened out and separated from each other. The preparation is then fixed for one minute in methyl alcohol and stained with the following mixture:

| | |
|---|---|
| Saturated alcoholic solution of rosanilin violet. | 2 drops |
| Saturated watery solution of methylene blue.. | 1 drop |
| Distilled water........................... | 10 c.c. |

If the stain is not sufficiently deep, double quantities of the dyes can be used. The mixture should always be freshly prepared from the stock solutions and then poured over the slide. The latter is then gently steamed over a flame, and after two minutes washed with water, dried, and examined with an oil immersion lens. The Negri bodies take a deep red color; their Chromatin granules stain bluish. The body of the nerve cell also takes a bluish stain.

Smears made in this manner may also be stained, after fixation in methyl alcohol, by means of the Giemsa stain as given on page 86. The bodies stain a clear robin's egg blue, the nuclei a deep purple, while the granular body of the nerve cell is pure dark blue.

## X. KALA AZAR (Tropical Splenomegaly)

Kala azar is a chronic febrile disease of tropical regions, characterized by progressive enlargement of the spleen and liver. A parasite (Fig. 180) has been found in the pulp of the spleen and liver, and in the lymph nodes, lungs and submucosa of the intestine, and very rarely in the blood, which is presumably the inciting agent of the disease. These Leishman-Donovan parasites, as they have been named, are usually found in the large mononuclear cells, in the phagocyting endothelial cells of the tissues especially the spleen, or, occasionally in the leucocytes. Very few extracellular parasites are found.

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[1] *Van Gieson:* Cent. f. Bakt., I. Abt., Orig., Bd. xliii, 1907, p. 205.

The parasites are small, round, or oval masses of protoplasm which stain faintly with basic stains, and contain two chromatin masses which stain deeply with ordinary basic stains and the chromatin stains. These bodies are of unequal size. The larger is oval and is situated at one side of the parasite, staining a pale red with methylene azure; the other is rod shape, and stains intensely red. The bodies are much the same size as the blood platelets.

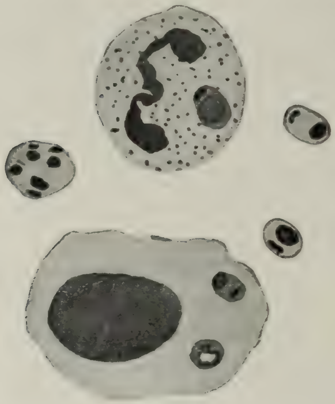

Fig. 180. — Parasites of Kala Azar from Splenic Puncture. × 2,000 diameters.

Similar bodies have been described by J. H. Wright in the exudate from a case of Delhi boil, and named Helcosoma tropicum. Nicolle has also found that in Italy and Northern Africa closely related parasites can be demonstrated in the splenic pulp of children suffering from acute splenic anæmia. This parasite grows in culture media and develops flagellate forms as does the Leishman-Donovan organism.

It has not yet been settled whether these three parasites all belong to the same species, and even the generic position of this group is still in question. The Leishman-Donovan parasite has been considered as an involution form of the Trypanosomata because of the flagellate bodies which develop in cultures. Other authors are inclined to class it with the Pyroplasmata, and still others as belonging to the Mastigophora or Flagellata.

These diseases are widely distributed in tropical countries. Kala-azar is endemic in northeastern India, and cases have been described in Ceylon, China, Arabia, Egypt, and the northern coast of Africa. The Oriental boil infection has much the same geographical distribution.

## XI. WASSERMANN REACTION

The theory of this test is as follows: When an antigen, that is, a substance which can induce the formation of an antibody, is placed in contact with inactivated serum containing the specific antibodies induced by this antigen, and also with fresh normal serum (complement) there is a union of the specific antibody with the antigen and this combination takes up the complement from the fresh normal serum. This binding of the complement is made evident by the fact that the combination after standing a suitable time is, in spite of its complement content, no longer able to bring into solution red corpuscles which have previously absorbed hæmolytic amboceptors (a so-called hæmolytic system), because before this hæmolysis can be induced the hæmolytic amboceptors must unite with free complement; the complement, however, having already been united with another amboceptor is not free to join with that of the charged red corpuscles. This reaction of *complement fixation* is quite a general one, and can be used to determine the presence of different proteids, or of bacterial products in the serum of a person suffering from an infectious disease, and recently has been applied in a very extensive manner to the determination of the presence or absence of certain antibodies found in the serum of persons suffering from syphilis.

The necessary apparatus includes small sterilized test tubes, 8 to 15 mm. in diameter and 10 cm. in length, pipettes accurately graduated to 0.01 and 0.1 c.c. and sterilized by heat, 0.85 per cent. sodium chloride solution, a thermostat running at 37° C., an apparatus in which water can be kept at a temperature of 55° C., a centrifuge with a speed of 1,000 revolutions per minute, and an icebox. In addition there is required:

**A. Patient's Serum.**—This is best obtained from one of the cubital veins by means of a hollow needle with a bore of 1 to 2 mm. connected with two inches of rubber tubing. A rubber tourniquet or bandage is fastened about the upper arm, tightly enough to constrict the veins and cause them to distend distally. The skin over the veins is carefully cleansed with soap, ether, and alcohol, and the needle (sterilized by boiling in 1% sodium carbonate) is plunged into the most prominent vein. Five to

15 c.c. of blood are allowed to flow into a sterile test tube, the tourniquet loosened, the needle withdrawn, and the puncture covered with a piece of sterile gauze. The tube is slanted at an angle of 10° from the horizontal until the blood coagulates. If coagulated in an upright position the edges of the clot should be freed from the surface of the tube with a platinum needle.

If a small needle is used it is of advantage to connect it with a syringe of 10 c.c. capacity so that gentle aspiration can be used. In infants a few punctures are made in the heel or great toe with a small, very sharp scalpel, and the blood allowed to drop into a Petri dish. After several hours the dish is tilted to one side and the clear serum drained off the surface of the clot. If necessary, the blood may be obtained by pricking the finger tip and constricting the finger at the base, or from the lobe of the ear, the blood in this instance being received into a centrifuge tube or several Wright capsules (Fig. 178, page 674). A fairly accurate test can be made with 0.1 c.c. of clear serum (about 0.5 c.c. of blood), but it is far more satisfactory to work with larger amounts.

After complete coagulation the blood is kept on ice—preferably for not more than five days as it may develop anticomplementary bodies. The serum is decanted or transferred with a capillary pipette (page 674) to a small test tube, which is then tightly plugged with cotton. If clouded with red cells it should be centrifugalized until clear.

*Inactivation.*—It is generally recommended that the complement in the serum be destroyed by heating, though in certain modifications of the Wassermann technique this is avoided (see page 700). The tubes containing the serum are placed in a double-wall water bath at 54° to 55° C. This is conveniently arranged by placing a small beaker in a "fish kettle" or by supporting it in an ordinary metal water bath on a pipe-stem triangle. Noguchi considers that a temperature of 56° C. is injurious, and that on no account should the serum be heated to 60°. The heating is continued for one-half hour. After inactivation the serum is returned to the icebox, and will often remain serviceable for months.

**B. Complement.**—Guinea-pig blood is obtained either by bleeding the animal to death or by aspirating the heart. In the former case the neck is clipped, the animal is anæsthetized with ether, and the carotid arteries are dissected free and severed. The blood is received into centrifuge tubes. After bleeding has stopped more blood can usually be obtained by holding the animal's head down and compressing the abdomen and thorax. When through bleeding the heart should be exposed and cut, as the animal might revive. Blood may also be obtained without killing the animal, with an aspirating syringe which is clean and dry. The animal is etherized; the chest is shaved, and the needle is introduced through the second or third right interspace close to the sternum, in the direction of the heart apex—downward, backward, and to the left to a depth of 1 to 2.5 cm. If the auricle is successfully entered, the blood will flow rather readily into the syringe on gentle aspiration. A guinea-pig of about 650 gms. weight will survive the loss of 6 to 8 c.c. of blood provided the heart is not lacerated by unsuccessful punctures, and may be bled again after about two weeks. The same animal should yield 10 to 15 c.c. if bled from the carotid. In bleeding from the heart it is better to take small amounts from several pigs than to make repeated punctures in one. Moreover, mixed blood specimens have more constant complementary values.

The blood thus obtained is allowed to clot, and the clot is promptly freed from the sides of the tube with a needle; it is then placed on ice for six to twenty-four hours, which increases its activity. The serum is then pipetted off, if necessary cleared by centrifugalization, and kept on ice until used. It may be preserved for two to three days, but should not be used after this. Immediately before use the amount needed is diluted one part of serum with nine parts of sterile 0.85 per cent. sodium chloride solution. Schemes for preserving complement, such as freezing the serum or drying it on filter paper, have not proved satisfactory, and have for the most part been discarded.

Practice varies in different laboratories as to the standardization of complementary sera. Some workers use a uniform amount, 0.1 c.c. as a unit. As the actual unit for a standardized amboceptor may vary from 0.05 c.c. to 0.15 c.c., it is evident that this procedure often leads to embarrassment. A deficient amount of complement may give incomplete hæmolysis in the controls, or an amount just sufficient to hæmolyze in the controls may give false positive reactions. On the other hand, an excess may mask a weak positive reaction and make it appear negative. These difficulties are diminished by using mixed serum from at least three guinea-pigs, but it is safer to standardize each complement before making the tests. It takes about five minutes to set up the tubes for this titration, and they may be incubated while the first steps of the final reaction are being made.

This titration may be done by using various amounts of amboceptor serum according to the protocol (page 695). As the variable factor is the guinea-pig serum, it seems more logical to titrate this against a constant amount of amboceptor. To avoid waste of reagents, all preliminary titrations are set up with one-fourth the amount of each reagent used in the Wassermann reaction. Small test tubes 8 mm. in diameter are convenient for this.

TITRATION OF COMPLEMENT (ONE-QUARTER UNITS).

| Tube No. | Guinea-pig Serum, 1-10. | Salt Solution. | Immune Rabbit Serum, 1 c.c. = 2 units, e.g., 1-800 dilution | Sheep Corpuscles, 5%. | | Hæmolysis | Corresponding Amount of Guinea-pig Serum for full units. |
|---|---|---|---|---|---|---|---|
| | c.c. | c.c. | c.c. | c.c. | | | c.c. |
| 1 | 0.38 | 0.5 | 0.13 | 0.25 | Incubate 1 hour at 37° C. | Complete | 0.15 |
| 2 | 0.30 | 0.58 | 0.13 | 0.25 | | Complete | 0.12 |
| 3 | 0.25 | 0.62 | 0.13 | 0.25 | | Complete | 0.1 |
| 4 | 0.20 | 0.67 | 0.13 | 0.25 | | Partial | 0.08 |
| 5 | 0.13 | 0.74 | 0.13 | 0.25 | | Slight | 0.05 |
| 6 | 0.06 | 0.81 | 0.13 | 0.25 | | None | 0.025 |
| 7 | — | 0.87 | 0.13 | 0.25 | | None | Control |

If the result were as given, 0.1 c.c. of serum (or 1 c.c. of the 1 to 10 dilution) being the smallest amount of guinea-pig serum which gave complete hæmolysis, would be the amount used in the test. When hæmolysis is complete in the fifth or sixth tubes, it is well to use a slight excess, 0.06 or 0.07, or 0.03, as these very active sera seem to lose some of their activity by incubation in the first stage of the Wassermann reaction.

Different specimens of serum vary in their fixability as well as in their hæmolytic activities, as has been noted by Stern [1] and by Noguchi.[2] In some laboratories a preliminary titration is made, using varying amounts of complement against a known positive serum and antigen. This is not generally regarded as necessary.

C. **Sheep Corpuscles.**—The blood may be obtained directly from the neck of a slaughtered sheep at an abattoir. If a stock animal is kept it is bled through a needle like that used in obtaining the patient's blood, but preferably of 2 to 3 mm. bore. The superficial veins about the knee or shoulder are suitable, or it is usually easy to tap the jugular after tying a rubber tourniquet about the root of the neck. The parts must be clipped and shaved. The blood is received directly into a more than equal amount of citrate solution (sodium citrate, 10 gms., sodium chloride, 8.5 gms., water, 1,000 c.c.) or into a flask containing beads, which is then vigorously shaken. Or the shed blood is whipped with wires to defibrinate it. It is then centrifugalized and the supernatant fluid is pipetted off. Five to ten volumes of 0.85 per cent. sodium chloride solution are added to the cells and mixed by drawing up and down in a pipette. The mixture is then centrifugalized until the supernatant fluid is clear, and the fluid is pipetted off. This procedure is repeated twice, so that the cells are three times washed with the salt solution. The sediment is then taken up in a graduated pipette and added to nineteen volumes of the salt solution (5 per cent. suspension). In this dilution the cells will keep two to four days, but should not be used if older than this or if there is any trace of dissolved hæmoglobin in the supernatant fluid.

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[1] *Stern:* Ztschr. f. Immunitätsforschung, Orig., 1910, v, p. 201.

[2] *Noguchi:* Jour. Exper. Med., 1911, xiii, p. 69.

**D. Antigen.**—Inasmuch as in syphilis it is impossible to obtain a pure culture of the specific organism, Wassermann proposed that the livers of fœtuses dead from syphilis, which contain great numbers of spirochetes, should be employed. He was successful with aqueous extracts of such livers; but it was soon shown that normal liver and other organs could be used, so that it was not the spirochetes, or at any rate not the spirochetes alone, that produced the reaction. Levaditi showed that purified lecithin and sodium glycocholate and taurocholate may also to a certain extent replace liver extracts. The extracts at present employed are not in the strict sense syphilitic antigens, but the term is retained for convenience.

An innumerable variety of extracts and mixtures have been suggested for this purpose, of which the following are the representative types. It is an advantage to set up tests in duplicate or triplicate with different kinds of antigen as the results not infrequently vary. The writer would suggest 2, 3, and 4 as preferable, in the order named.

### AQUEOUS EXTRACTS

*1. Wassermann's Method.* The liver of a syphilitic fœtus is minced in a meat grinder or ground in a mortar with quartz sand. It is mixed with salt solution containing 0.5 per cent. phenol in the proportion of 1 gram to 4 c.c., and the mixture is shaken for twenty-four hours in a shaking machine. It is then centrifugalized at low speed, or better, allowed to settle and decanted. The brownish, slightly turbid fluid is kept in the dark on ice. A sediment forms which should not be disturbed. Before use it must be standardized by the general method given for all extracts. It should be active in doses of not over 0.2 c.c. It usually shows a slight degree of inhibition with normal sera, and does not give as sharp readings as other preparations. The aqueous extract is very unstable. It may remain serviceable for some time or may lose its activity over night. If it is to be preserved for any length of time it should be dried in a vacuum.

### ALCOHOLIC EXTRACTS

*2. From Syphilitic Material* (Bruck [1]). The liver of a luetic fœtus is cut fine and placed in a bottle with glass beads. Nine c.c. of absolute alcohol are added for each gram of liver. The mixture is shaken for twenty-four hours in a shaking machine, and filtered through paper. (Some workers dispense with the shaking machine.)

This extract, according to Bruck, is useful in amounts of from 0.2 to 0.3 c.c., even if somewhat hæmolytic of itself, as serum prevents the hæmolytic action. It must not be anticomplementary in the doses used, but need not be tested in double the dose.

*3. From Normal Material* (Landsteiner [2]). Guinea-pig heart, freed from fat, is ground fine and 5 c.c. of 95 per cent. alcohol added for each gram of material. The mixture is heated for ten to twelve hours at 60° C. and filtered through paper. It may be preserved in the dark at room temperature indefinitely. It is useful in doses of from 0.3 to 0.05 c.c.

Normal human heart extracted in this way is useful and similar extracts of guinea-pig, human, or beef liver, made at 60° or 22° are recommended by various workers.

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[1] *Bruck :* Die Serodiagnose der Syphilis, Berlin, 1909, p. 27.

[2] *Landsteiner, Müller* and *Pötzl:* Wien klin. Wchnschr., 1907, xx, p. 1565.

### FRACTIONATED LIPOIDS

*4. Noguchi's Method.* Noguchi,[1] studying the properties of various fractions of lipoids from liver extracts, found that the antigenic substances were chiefly in the fraction soluble in ether and alcohol, but insoluble in acetone. This fraction contains lecithin and other phosphatids. He recommends an antigen prepared in the following way:

Extract minced syphilitic (or normal) liver, heart or kidney from man, ox, guinea-pig, rabbit or dog, with 10 volumes of 95 per cent. alcohol for six or seven days at 37° C., shaking occasionally. Filter through paper, and evaporate in crystallizing dish under a fan (or in vacuum) at a temperature below 40° C. Take up the dry residue with ether and allow the turbid solution to stand in a covered dish over night. Decant the clear supernatant portion, and concentrate by evaporating the greater part of the ether. Add the concentrated ethereal solution to ten volumes of acetone, causing a waxy mass to precipitate. Dissolve 0.3 gram of this precipitate in 1 c.c. of ether and add 9 c.c. of methyl alcohol. The precipitate is removed by sedimentation. This alcoholic solution is quite permanent, and an aqueous emulsion is prepared each time before making the test by diluting the stock extract with 9 volumes of saline solution. One-tenth of a c.c. of this 0.3 per cent. emulsion is used in the test.

This would seem *a priori* the best of the extracts suggested, and Noguchi claims that it is the only one which may be used with active sera. In the writer's hands similar antigens, prepared by Noguchi's earlier method, gave a slightly smaller percentage of positive reactions than did simple alcoholic extracts.

### ARTIFICIAL MIXTURES

5. None of the artificial mixtures suggested seem as efficient as the organ extracts. Sachs and Rondoni devised the following:

| | Mixture A | Mixture B. |
|---|---|---|
| Sodium oleate (Kahlbaum) | 2.5 | 1.0 |
| Lecithin (ovo) (Merck) | 2.5 | 1.0 |
| Oleic acid (Kahlbaum) | .75 | 1.5 |
| Distilled water | 12.5 | 5.0 |
| Alcohol, ad | 1,000. | 1,000. |

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[1] *Noguchi:* Serum Diagnosis of Syphilis, Philadelphia, 1911.

The alcoholic solution is diluted 1 to 5 with salt solution, and from 0.15 to 0.4 c.c. are used in the test.

Even according to the authors the mixtures give less strong inhibition than organ extracts.

*Standardizing Antigens.* Before an extract can be used as antigen it must be determined in what amount it is lytic of itself and in what amount it is anticomplementary. As a general rule, the amount used in the reaction should be half that which causes no inhibition of itself; for should a normal serum (as is sometimes the case) inhibit half the complement and the antigen inhibit half, we might get complete inhibition by summation without any interaction between antigen and serum. This hypothesis is somewhat theoretical and is neglected by some workers who use alcoholic antigens; but on the whole the rule is a safe one. The following protocol explains the method of titration. (Quarter units are again used, page 688.)

Before testing a dilution should be made by adding three parts of salt solution drop by drop to one part of extract.

If the result were as given (p. 693), 0.2 c.c. would be the largest amount which gave no inhibition. One-half of this (0.1) does not hæmolyze at all of itself and may be used in the test, and is considered the unit. Before use enough antigen is taken from the stock bottle for the number of reactions to be done and diluted with salt solution so that 1 c.c. equals 1 unit. (In this case, 1 part of extract to 9 of salt solution.) In the case of alcoholic extracts the salt solution should be added to the stock solution drop by drop, shaking between drops. The method of dilution has a marked effect on the antigenic power of the extract.

The hæmolytic and anticomplementary powers being determined, the extract must be further tested as to its antigenic properties. Different preparations made in an identical manner will be found to differ widely and some will be absolutely unserviceable. The extract may be tested by setting up a series of tests with 10 to 20 known positive and 10 to 20 known negative sera. It is more convenient to use it in parallel with an antigen of known quality, doing all new reactions for some time with both extracts. If the results agree it may then be substituted for the old extract. In doing the reaction for the first time a control antigen should be secured which has been tried out in another laboratory. If this is impossible the extract should be tested against serum from a

## TITRATION OF HÆMOLYTIC ACTIVITY OF EXTRACT (ONE–QUARTER UNITS)

| Tube No. | Extract. | Salt Solution. | Sheep Corpuscles, 5%. | | Hæmolysis. | Amount of Extract for Whole Unit. |
|---|---|---|---|---|---|---|
| | c.c. | c.c. | c.c. | | | c.c. |
| | Undiluted | | | | | |
| 1 | 0.2 | 0.8 | 0.25 | Incubate 1 hour at 37° C. | Complete | 0.8 |
| 2 | 0.15 | 0.85 | 0.25 | | Complete | 0.6 |
| | Extract 1–4 | | | | | |
| 3 | 0.4 | 0.6 | 0.25 | | Partial | 0.4 |
| 4 | 0.3 | 0.7 | 0.25 | | Partial | 0.3 |
| 5 | 0.2 | 0.8 | 0.25 | | Slight | 0.2 |
| 6 | 0.16 | 0.84 | 0.25 | | None | 0.16 |
| 7 | 0.1 | 0.9 | 0.25 | | None | 0.1 |
| 8 | 0.06 | 0.94 | 0.25 | | None | 0.06 |
| 9 | 0.04 | 0.96 | 0.25 | | None | 0.04 |
| 10 | 0.0 | 1.0 | 0.25 | | None | Control |

## TITRATION OF ANTICOMPLEMENTARY POWER OF EXTRACT (ONE–QUARTER UNITS)

| Tube No. | Extract. | Salt Solution. | Guinea-pig Serum, 1–10. | | Activated Corpuscles[1] (2 amboceptor units). | | Hæmolysis. | Amount of Extract for Full Unit. |
|---|---|---|---|---|---|---|---|---|
| | c.c. | c.c. | c.c. | | c.c. | | | c.c. |
| | Undiluted. | | | | | | | |
| 1[3] | 0.2 | 0.3 | 0.25[2] | Incubate 1 hour at 37° C. | 0.5 | Incubate 1 hour at 37° C. | Complete | 0.8 |
| 2[3] | 0.15 | 0.35 | 0.25 | | 0.5 | | Marked | 0.6 |
| | Extract 1–4 | | | | | | | |
| 3 | 0.4 | 0.1 | 0.25 | | 0.5 | | None | 0.4 |
| 4 | 0.3 | 0.2 | 0.25 | | 0.5 | | Marked | 0.3 |
| 5 | 0.2 | 0.3 | 0.25 | | 0.5 | | Complete | 0.2 |
| 6 | 0.16 | 0.34 | 0.25 | | 0.5 | | Complete | 0.16 |
| 7 | 0.1 | 0.4 | 0.25 | | 0.5 | | Complete | 0.0 |
| 8 | 0.06 | 0.44 | 0.25 | | 0.5 | | Complete | 0.06 |
| 9 | 0.04 | 0.46 | 0.25 | | 0.5 | | Complete | 0.04 |
| 10 | 0.0 | 0.5 | 0.25 | | 0.5 | | Complete | Control |

[1] See under Amboceptor, page 696.

[2] Or one-fourth the amount determined by titration as unit, page 688.

[3] In working with alcoholic extracts, Nos. 1 and 2 need not be set up, as the serum would be coagulated.

frank case of secondary syphilis and against normal serum; if it gives complete inhibition with the former and none with the latter it may be depended on with a fair degree of safety. It is not necessary to standardize the antigen anew each time a reaction is done.

In standardizing extracts prepared by method 4, Noguchi uses only three tubes, one containing 0.4 c.c. of the 0.3 per cent. emulsion and corpuscles, to determine the hæmolytic property; another containing 0.4 c.c. of the emulsion, a full unit of complement, and later sensitized corpuscles, to test the anticomplementary property; and the third containing 0.02 c.c. of the emulsion, the syphilitic serum, complement, and later sensitized corpuscles, to determine its antigenic power. In other words, he requires that the antigen shall not be hæmolytic or anticomplementary in four times the amount used in the test, and shall give a positive reaction with known syphilitic serum in one-fifth of the amount used in the test. About 50 per cent. of the emulsions prepared by his method fulfil these requirements.

**E. Hæmolytic Serum.**—The hæmolytic serum is obtained from rabbits which have been injected with sheep corpuscles. The cells are obtained as described on page 688, and washed thoroughly three times in salt solution, using sterile glassware and sterile solutions. If the blood is contaminated, as is usually the case when it is obtained from a slaughterhouse, it is well to sterilize the cells at 60° C. for one-half hour, which does not destroy their antigenic properties. On account of the uncertainties of the method it is well to start three rabbits at once. Injections are best made intraperitoneally (page 253) at intervals of about 5 days. Four injections of 5, 10, 18, and 30 c.c. of a 50 per cent. suspension of cells are usually sufficient; some writers recommend smaller doses, increasing from 2 to 12 c.c. The same doses may be given subcutaneously but are apt to produce abscesses. Many writers recommend injections into the ear vein. In this case smaller doses, 1 to 2 c.c. of a 10 per cent. suspension (not increasing above this), are used. The second or third injection frequently causes sudden death due to the presence of agglutinins (anaphylaxis?). It is often satisfactory to start animals with one or two intravenous injections and continue the immunization intraperitoneally. Ten days after the fourth injection a sample of blood is taken from the ear vein and the serum is titrated. The serum is first inactivated, as described under patient's serum (page 686). One-tenth of a c.c. is carefully measured out with a pipette graduated in $\frac{1}{1000}$ths c.c. and *thoroughly* mixed with 30 c.c. of salt solution. One c.c. of this dilution is further diluted with 9 c.c. of salt solution. If the serum is lytic in dilution of 1 to 800 or more, the animal is anæsthetized and a glass cannula with a short rubber tube is inserted into the abdominal aorta (or carotid or femoral artery), and the blood is collected in a series of centrifuge tubes. This should be done under sterile precautions. The clots are freed from the sides of the tubes, and then allowed to contract in the cold over night, and the serum pipetted off. The last traces of serum are expressed by centrifugalization. The serum is inactivated and placed in a series of small sterile tubes which are sealed with a blast lamp or closed with air tight stoppers, and stored on ice in the dark. It keeps indefinitely, but it is best to titrate the serum whenever a new tube is broached, as it may deteriorate slowly.

If the preliminary titration shows the serum to be unsatisfactory, another injection of 20 to 30 c.c. should be given.

TITRATION OF HÆMOLYTIC SERUM (ONE–QUARTER UNITS)

| Tube No. | Immune Rabbit Serum. | Salt Solution. | Guinea-pig Serum 1–10. | Sheep Corpuscles, 5% Suspension. | | Hæmolysis. | Corresponding Dilution for Whole Units. |
|---|---|---|---|---|---|---|---|
| | c.c.<br>1–300 | c.c. | c.c. | c.c. | | | |
| 1 | 0.75 | 0.25 | 0.0 | 0.25 | Incubate 1 hour at 37° C. | None | Control |
| 2 | 0.75 | 0.00 | 0.25[1] | 0.25 | | Complete | 1/100 |
| 3 | 0.25 | 0.50 | 0.25 | 0.25 | | Complete | 1/300 |
| 4 | 0.13 | 0.62 | 0.25 | 0.25 | | Complete | 1/600 |
| 5 | 0.09 | 0.66 | 0.25 | 0.25 | | Complete | 1/800 |
| | 1–3000 | | | | | | |
| 6 | 0.75 | 0.00 | 0.25 | 0.25 | | Complete | 1/1000 |
| 7 | 0.63 | 0.12 | 0.25 | 0.25 | | Complete | 1/1200 |
| 8 | 0.47 | 0.28 | 0.25 | 0.25 | | Complete | 1/1600 |
| 9 | 0.38 | 0.37 | 0.25 | 0.25 | | Marked | 1/2000 |
| 10 | 0.30 | 0.45 | 0.25 | 0.25 | | Marked | 1/2500 |
| 11 | 0.25 | 0.50 | 0.25 | 0.25 | | Slight | 1/3000 |
| 12 | 0.00 | 0.75 | 0.25 | 0.25 | | None | Control |

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[1] Guinea-pig serum should first be titrated with a known hæmolytic serum to determine the unit quantity, or a mixture from several pigs should be used.

The smallest amount which produces complete hæmolysis is regarded as the unit, in this case 1 c.c. of a 1 to 1,600 dilution. This is the amount used in titrating complement. In performing the reaction, two units are used (in the case given, a 1 to 800 dilution). Before being added to the tubes the diluted serum is mixed with an equal volume of 5 per cent. corpuscles, and incubated at 37° C. for fifteen to sixty minutes. The mixture is termed "activated corpuscles."

## TECHNIQUE OF THE REACTION

The glassware used in the test must be clean and dry, but not necessarily sterile. The stock hæmolytic serum and other sera which are to be kept for some time are the only reagents which require sterile conditions. The pipettes, if used for more than one reagent, should be rinsed well in salt solution before a new reagent is drawn up. After using they should be placed in a cylinder of water so that the serum will not dry in them. After use they should be thoroughly cleaned, then soaked for several hours in clear water and dried in the oven in a position in which they will drain.

The various reagents being prepared and standardized, one is ready to proceed with the test. The tubes are conveniently set up in a double row, the front row containing antigen, the back row the control without antigen. If several antigens are used a separate row is set for each extract. Two-tenths c. c. of patient's serum, 1 c.c. of extract (diluted so that each c.c. contains 1 unit), and 1 unit of guinea-pig serum are first mixed and incubated for one hour to induce the combination of substances in the extract and serum which will bind the complement. At the end of one hour the activated cells are added, and the mixture is again incubated for one hour to determine whether the complement has been bound or is free to act.

If the cells are hæmolyzed the pink opaque fluid becomes deep crimson and perfectly transparent; if there is no hæmolysis the cells gradually settle to the bottom of the tube, leaving the supernatant fluid clear and colorless. Slight degrees of laking are recognized by the red color of the supernatant fluid after sedimentation, or by holding the tube toward the light side by side with a control tube of intact cells in the same dilution. If a wire rod or pencil is then placed across the far side of the two tubes it will throw a sharper shadow through the tube in which hæmolysis has begun.

If there is no hæmolysis or only a trace of hæmolysis at the end of an hour, the reaction is regarded as positive. If there is complete hæmolysis, it is negative. If there is considerable hæmolysis but enough cells remain intact to make the tube distinctly opaque, the result is considered a doubtful positive. Such sera are usually, but not invariably, syphilitic; they are frequently obtained from patients who have undergone treatment but are not cured. Slight degrees of inhibition are of no definite significance, but an absolutely negative report should not be made in such cases.

The following control tubes must be set up simultaneously: 1. A tube containing 0.4 c.c. of the patient's serum but no antigen (the "back row"). 2. Two tubes containing 0.2 and 0.4 c.c. of a known positive serum, with and without antigen, respectively, to prove that the antigen is efficient. 3. Similar controls with a known negative serum to prove that the antigen will not give "false positives." 4. A tube containing complement and two units of antigen, but no serum (and with alcoholic antigens, another tube with one unit of antigen) to prove that the antigen will not inhibit of itself. 5. A tube containing complement only, to prove that the hæmolytic system is efficient. 6. A tube containing one unit of antigen only, to prove that none of the reagents are lytic without complement. All these controls are brought up to the same volume (3 c.c.) with salt solution, and incubated for one hour. Two c.c. of a suspension of activated cells is then added, and the tubes again incubated. All the controls must show complete hæmolysis, except No. 6 and the one containing positive serum and antigen. These two must show no hæmolysis. If any of the controls except the first give unexpected results, it shows that one of the reagents is defective. A certain number of sera will show some degree of inhibition in this first control. If the front row tube is hæmolyzed, the result may still be considered negative, but a positive reaction with any inhibition in the back row must be viewed with suspicion. If this inhibition is at all marked, no reading of positive or negative can be made. This occurs in sera that contain hæmolyzed blood, in those which have been kept too long before inactivation, in other old sera, especially those in which there is bacterial growth, in sera which have been inactivated at too high a temperature, and in other unexplained conditions. The only way to obtain a result in such cases is to take a fresh sample of blood and test it immediately, setting up the test with both active and inactivated sera.

According to the original method duplicate tests were made with 0.1 as well as with 0.2 c.c. of the patient's serum. Controls were also set up with each of the sera under examination as well as with the known positive and known negative specimens, using aqueous extract of normal liver, which should all hæmolyze. There is, of course, no occasion for such controls in using alcoholic extracts, and they are unnecessary even if an aqueous antigen is used.

The following protocol will make the details sufficiently clear. Before the tubes are incubated the first time they should be shaken by inverting several times with the finger held over the end of the tube. The finger should be wiped on a moist towel after shaking each tube. After adding the hæmolytic system, shaking in a vertical position is sufficient.

To incubate the tubes, the rack should be placed in a water bath (fish kettle) at a temperature of 37° C. After ten minutes, when they have assumed the temperature of the water, they may be transferred to an air incubator if desired.

If the amount of serum is insufficient for the reaction as given, it may be set up in quarter units, using 0.05 c.c. of serum in the test, and the same amount in control, and using one-quarter the amount of all the other reagents given.

### USE OF OTHER BODY FLUIDS

Cerebrospinal fluid gives a positive reaction in 0.2 c.c. in most cases of paresis and about 50 per cent. of cases of tabes, and in a few cases of cerebrospinal syphilis. It contains no complement and need not be inactivated. By using 0.8 c.c., Hauptmann[1] obtained positives in 7 out of 12 cases of tabes and in 11 out of 14 cases of cerebrospinal syphilis, all of which were negative in 0.2 c.c. Twelve normal cases and 26 cases with other cerebrospinal lesions (including 6 of multiple sclerosis) were all negative in this large dose. Some of these negative cases had syphilitic lesions elsewhere and gave a positive test in their serum.

Pleural and ascitic fluids from syphilitic patients usually react

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[1] *Hauptmann:* München. med. Wchnschr., 1910, lvii, p. 1581.

WASSERMANN REACTION

| | FRONT ROW. | | | | | | | | BACK ROW. | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Human Serum. | Guinea-pig Serum,[1] 1–10. | Extract,[2] 1–10. | Salt Solution. | | Activated Corpuscles.[3] | | Hæmolysis. | Human Serum. | Guinea-pig Serum,[1] 1–10. | Salt Solution. | | Activated Corpuscles.[3] | . | Hæmolysis. |
| | c.c. | c.c. | c.c. | c.c. | | c.c. | | | c.c. | c.c. | c.c. | | c.c. | | |
| 1. Unknown serum A | 0.2 | 1.0 | 1.0 | 1.0 | Incubate 1 hour at 37° C. | 2.0 | Incubate 2 hours at 37° C. | None | 0.4 | 1.0 | 2.0 | Incubate 1 hour at 37° C. | 2.0 | Incubate 2 hours at 37° C. | Complete |
| 2. " " B | 0.2 | 1.0 | 1.0 | 1.0 | | 2.0 | | Complete | 0.4 | 1.0 | 2.0 | | 2.0 | | Complete |
| 3. " " C | 0.2 | 1.0 | 1.0 | 1.0 | | 2.0 | | Partial | 0.4 | 1.0 | 2.0 | | 2.0 | | Complete |
| 4. " " D | 0.2 | 1.0 | 1.0 | 1.0 | | 2.0 | | None | 0.4 | 1.0 | 2.0 | | 2.0 | | Partial |
| 5. Positive control | 0.2 | 1.0 | 1.0 | 1.0 | | 2.0 | | None | 0.4 | 1.0 | 2.0 | | 2.0 | | Complete |
| 6. Negative " | 0.2 | 1.0 | 1.0 | 1.0 | | 2.0 | | Complete | 0.4 | 1.0 | 2.0 | | 2.0 | | Complete |
| 7. Hæmolytic system | 0 | 1.0 | 2.0 | 0 | | 2.0 | | Complete | 0 | 1.0 | 2.0 | | 2.0 | | Complete |
| 8. " " | 0 | 1.0 | 1.0 | 1.0 | | 2.0 | | Complete | ... | ... | ... | | ... | | ...... |
| 9. Color control | 0 | 0 | 1.0 | 2.0 | | 2.0 | | None | ... | ... | ... | | ... | | ...... |

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[1] The amount in this column is varied according to titration.

[2] Dilution of extract is varied according to titration.

[3] Made by mixing a 5-per-cent. suspension of washed cells with equal parts diluted serum, containing 2 units per c.c. In this case a 1 to 800 dilution.

The results of such a test would be:

| | |
|---|---|
| Serum A, positive. | Serum C, doubtful positive. |
| Serum B, negative. | Serum D, no reading possible. |

positively in 0.2 c.c., though less strongly than blood serum. Statistics are, however, not large enough for results with these fluids to be reliable. They contain little or no complement.

Fluid from fly blisters acts like serum,[1] but saliva and tears do not give the reaction.

Milk from syphilitics gives a reaction.[2]

Positive results with urine in amounts of 0.5 to 1 c.c. have been reported,[3] but this fluid is often strongly anticomplementary.

### MODIFICATIONS IN TECHNIQUE

Innumerable modifications of the complement fixation test for syphilis have been devised with a view to simplifying the technique or making the reaction more delicate or more accurate. In order to work with small amounts of serum, it has been suggested to measure the reagent by drops (Landsteiner, R. Müller, and Noguchi), or to set up the reaction in capillary pipettes (Weidanz). To avoid errors due to the presence of amboceptor for sheep cells in human blood, Noguchi and Tschernogubow have used human cells, and Browning, ox cells. Bauer utilizes the human antisheep amboceptor and dispenses with the immune rabbit serum. Stern utilizes human complement and dispenses with the guinea-pig serum. Hecht dispenses with both rabbit and guinea-pig blood. Special advantages are claimed for each modification, but up to the present no one has obtained general acceptance or has to any extent replaced the Wassermann technique. The systems in which active human serum is employed give a higher percentage of positives, but it has not been established that sera which give the test only when active are always syphilitic. Certainly, attempts to increase the delicacy by diminishing the amount of complement, etc., used are to be discouraged as they almost invariably lead to false positive reactions. As the reaction is in itself empirical, no modification is of value until large statistics have been collected. The original technique is of far greater value than any modification on account of the immense number of reactions recorded by this method. Some of the more important modifications are given below.

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[1] *Borelli u. Messineo:* Biochem. Zentralbl., 1909, viii, p. 882.

[2] *Thomsen:* Berl. klin. Wchnschr., 1909, xlvi, p. 2052.

[3] *Blumenthal u. Wile:* Berl. klin. Wchnschr., 1908, xlv, p. 1050.

### *Noguchi's*[1] *Modification*

Most adult human sera contain lysin for sheep corpuscles which varies greatly in amount. If present in excess it may so increase the amount of amboceptor in the original Wassermann that hæmolysis will occur even after most of the complement has been bound, and a reaction which should be weakly positive may be converted into a false negative. To avoid this, Noguchi uses human blood cells and the serum of rabbits immunized against them. Moreover, he uses active serum which, as has been said, increases the number of positives. He also employs small amounts of reagents, measuring the fresh sera, etc., by drops, and the amboceptor and antigen by drying them on paper which is then cut into uniform squares. This is not an essential feature in the technique, and there is no *a priori* reason why the human-rabbit hæmolytic system and active syphilitic sera should not be employed in the proportions used in the Wassermann reaction. This system has very distinct theoretical advantages over the original method, and in the hands of Noguchi and others is just as specific. The immunization of rabbits to human cells is for some reason far more difficult than that to sheep cells. This may explain why the modification is not more generally used.

The following reagents are required:

1. Antihuman hæmolytic amboceptor, prepared by injecting rabbits with increasing doses of washed human corpuscles intraperitoneally. These are best obtained from the maternal end of the umbilical cord immediately after delivery, from venesection, or at autopsies performed soon after death. Five doses of 4, 7, 10, 15, and 15 to 20 c.c. (the amounts refer to the original volume of blood) are given at five-day intervals, and in nine days a preliminary test is made (page 695). The animal is then bled, the serum is inactivated and poured over sheets of filter paper (e.g. Schleicher u. Schüll No. 597) in a flat dish. When the paper is saturated the excess is absorbed by a dry sheet and the paper spread out on unbleached muslin to dry. When dry it is cut into strips 5 mm. wide and kept dry and sealed in the dark. To titrate, a series of tubes are set up, each containing 1 c.c. of a 1 per cent. suspension of human erythrocytes and 0.02 c.c. of guinea-pig serum. To these are added increasing lengths of ambo-

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[1] *Noguchi:* Jour. Exper. Med., 1909, xi, p. 392.

ceptor strips, 1 mm., 2 mm., 3 mm., etc. The shortest strip which produces complete hæmolysis in two hours is the unit.

2. Human Corpuscles. A 1 per cent. suspension of washed cells is used. This may be obtained by the addition of 1 drop of blood to 4 c.c. of salt solution, which is then centrifugalized (or allowed to settle), decanted, and restored to the original volume with salt solution.

3. As antigen Noguchi uses lipoids prepared by method No. 4. This may also be prepared in the form of slips. Four-tenths of a gram of the acetone precipitate is dissolved in 7 c.c. of ether. Filter paper is saturated with the solution, and dried like the hæmolytic serum. It is titrated like a liquid, using varying lengths of 5 mm. strips instead of varying fractions of a cubic centimeter, and human cells and antihuman amboceptor instead of sheep.

4. For complement, fresh guinea-pig serum is used diluted with one and one-half volumes of saline solution.

The test is performed with two rows of tubes:

| | Active Patient's Serum. | Guinea-pig Serum, 40%. | Antigen. | Washed Human Cells, 1%. |
|---|---|---|---|---|
| Front row..... | 1 drop (0.02 c.c.) | 0.1 c.c. | 1 unit | 1 c.c. |
| Back row...... | 1 " " | 0.1 " | None | 1 " |

The usual controls with positive and negative sera, etc., are also set up. After one hour's incubation two units of amboceptor are added and the tubes are incubated for two hours at 37° C. If the patient's serum is inactivated four drops instead of one are used in the test.

### *Stern's Modification*[1]

This consists in utilizing the complement in fresh human serum and dispensing with guinea-pig serum. Human complement is fairly constant in amount, though some sera show deficiency or excess. The amount is smaller than in guinea-pig serum and diminishes if the serum is not quite fresh. Stern uses a 2.5 per cent. suspension of sheep corpuscles, and nine to twelve units of sheep amboceptor. (If perfectly fresh sera are used, 2 to 3 units are sufficient.) To exclude anticomplementary action of the antigen, $\frac{1}{5}$ or $\frac{2}{5}$ the original amount is used. Three rows of tubes are set up:

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[1] *Stern:* Ztschr. f. Immunitätsforschung, 1909, i, p. 422.

| | Patient's Serum (active). | Antigen. | Salt Solution. |
|---|---|---|---|
| First row . . . . . . . . . . . . . . | 0.2 c.c. | $\frac{2}{5}$ unit | q.s. ad 2 c.c. |
| Second row . . . . . . . . . . . . | 0.2 " | $\frac{1}{5}$ " | " " 2 " |
| Third row . . . . . . . . . . . . . | 0.2 " | None | " " 2 " |

After one hour's incubation, add 1 c.c. of a 1 to 160 (or 10 units) immune rabbit serum, and 1 c.c. of a 2.5 per cent. suspension of sheep corpuscles.

This is a useful modification, but certain sera are almost devoid of complement and will not react even with excess of amboceptor. In Bruck's laboratory all sera are tested by both Wassermann's and Stern's methods. In 600 reactions, the latter gave a positive result only with luetic sera, and gave 15 per cent. more positives than did the older technique.

### *Bauer's Modification* [1]

This consists in utilizing the sheep amboceptor usually present in human serum. As this varies greatly in amount, and is lacking in all infants and many adults, the method is full of uncertainty. If used in parallel with the Wassermann, however, it affords certain positive reactions in syphilitic sera with so much amboceptor that the Wassermann gives negative results. (Jacobaeus' method would seem a more certain means for attaining this end.) The tubes are set up with inactivated serum exactly as in the Wassermann, except that 1 c.c. of a 5 per cent. suspension of sheep corpuscles are added instead of activated cells. If at the end of two hours, hæmolysis is incomplete, 0.1 or 0.2 c.c. (as seems necessary) of some negative serum which has completely hæmolyzed are added to the front and back row. It is rather better to add 1 or 2 units of inactivated rabbit serum.

Hecht's [2] modification is even more uncertain than Bauer's technique.

### *Wechselmann's Barium Sulphate Method*

Wechselmann [3] was able to increase his percentage of positive reactions with syphilitic sera by treating them with barium sulphate before testing. This removes substances from the serum (complementoid?) which interfere with binding of complement.

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[1] *Bauer:* Semaine méd., 1908, xxviii, p. 429.

[2] *Hecht:* Wien. klin. Wchnschr., 1909, xxii, p. 338.

[3] *Wechselmann:* Ztschr. f. Immunitätsforschung, Orig., 1909, iii, 524.

This phenomenon has been further studied by Noguchi,[1] and though not yet thoroughly tried out in practice promises to be of value.

A fresh 7 per cent. suspension of barium sulphate is prepared by precipitating 25 c.c. of a 5 per cent. barium chloride solution with dilute sulphuric acid, avoiding an excess of acid. The precipitate is then washed several times in a centrifuge tube with distilled water in the same manner as blood cells, and then suspended in 20 c.c. of distilled water. To 0.9 c.c., of the inactivated serum, 3 c.c. of salt solution and 0.5 c.c. of this barium sulphate suspension are added in a centrifuge tube, and the mixture is incubated for one hour at 37° C. and centrifugalized. One c.c. of the clear supernatant fluid is used for the test and 2 c.c. for the control.

### *Absorption of Antisheep Amboceptor*

Jacobaeus[2] has recently suggested that the sheep amboceptor in the patient's blood and also complementoid can be removed by digestion of the serum with sheep cells before testing. Seven-tenths of a c.c. of the inactivated serum are placed in a centrifuge tube with 2.8 c.c. of 5 per cent. sheep corpuscle emulsion, and incubated for half an hour. The mixture is then centrifugalized and 1 c.c. of the clear supernatant fluid is used for the test and 2 c.c. for the control. This preliminary treatment of the serum is especially valuable in quantitative work.

## QUANTITATIVE ESTIMATION OF THE WASSERMANN BODIES

The Wassermann reaction does not depend on the presence of a serum constituent peculiar to syphilis, but on an excess of substances present in smaller amounts in many or all normal sera. As has been stated, modifications which increase the sensitiveness of the reaction give positive tests with non-syphilitic sera. It is essentially a quantitative, not a qualitative, test, and it would be of the greatest advantage to be able to express the result quantitatively. On account of the great variability of the reagents employed and the interfering substances in the patient's blood itself, especially antisheep corpuscle amboceptor, an accurate quantitative expression is impossible with the present technique. We can, however, obtain results which are of value provided that when interpreting them we bear in mind the large errors which may occur.

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[1] *Noguchi:* Jour. Exper. Med., 1911, xiii, 217.

[2] *Jacobaeus:* Ztschr. f. Immunitätsforschung, Orig., 1911, viii, 615.

A quantitative expression may be obtained by simply measuring the unlaked sediment in a graduated Purdy tube, or better, by estimating the hæmoglobin in the supernatant fluid (after centrifugalization or sedimentation in the icebox). Boas[1] prepares a scale from the same corpuscle emulsion used in the test, by diluting it with four volumes of distilled water, which gives a 100 per cent. solution, and then making twenty-one serial dilutions ranging from 90 to 2 per cent. (A shorter scale of four or five tubes is more convenient though it lacks a certain pseudo-accuracy.) Boas in testing 1,064 sera from non-syphilitics found none which gave less than 70 per cent. hæmolysis. Consequently he regards any reading under 60 per cent. as positive. This method gives no difference between sera which completely inhibit in 0.2 c.c.

Other methods consist in setting up a series of tubes and varying the amount of patient's serum, or of antigen, or of complement used, or varying all three at once (Zeissler[2]). It seems more rational to use graded amounts of one reagent, preferably of patient's serum, 0.4, 0.2, 0.1, 0.05, 0.025, 0.01, keeping the other quantities constant. This is the best quantitative method we have at present. By means of it Boas was able to follow the gradual reduction of syphilitic antibody under specific treatment. Citron[3] uses simply 0.2 and 0.1 c.c., and expresses the results as follows:

| | | |
|---|---|---|
| No. 1 and No. 2 show complete inhibition | + + + + | Strong positive. |
| No. 1, complete inhibition; No. 2, incomplete inhibition | + + + | |
| No. 1 “ “ No. 2, no inhibition | + + | Weakly positive. |
| No. 1, incomplete “ No. 2, “ “ | + | |
| No. 1, doubtful “ No. 2, “ “ | ± | Doubtful. |
| No. 1 and No. 2 both completely hæmolyzed | — | Negative. |

In quantitative work the reagents must be standardized with special care.

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[1] *Boas:* Die Wassermannsche Reaktion, Berlin, 1911.

[2] *Zeissler:* Berl. klin. Wchnschr., xlvi, 1909, p. 1968.

[3] *Citron:* Die Methoden d. Immunodiagnostik u. Immunotherapie, Leipzig, 1910.

### RESULTS OBTAINED BY THE REACTION

As no theory explaining the reaction has been generally accepted, none will be given here. Suffice it to say that it is not a specific immunity phenomenon, inasmuch as the so-called antigen is a substance of lipoid nature which is a physiological body constituent and not a product of the Spirochæte pallida. The test is empirical (and in this sense should be classed with Ehrlich's diazo reaction rather than with the Widal), and its value must be determined by statistics. The results seem to show that it is of real diagnostic value if considered in connection with other clinical data. Positive results are occasionally obtained in certain conditions mentioned below. If these can be excluded, a positive Wassermann properly performed is almost absolute evidence of syphilis. It does not mean that the symptoms of which the patient complains are due to syphilis; the infection may be latent. A negative Wassermann, on the other hand, by no means excludes syphilis. A number of frank active cases, especially in the primary and tertiary stages, give negative results. Many patients giving a definite history of an old infection with slight symptoms suggestive of syphilis, give a negative test, although the symptoms respond promptly to specific treatment. Inasmuch as the reaction depends on an increase of substances probably normally present, to amounts in which they may be recognized in test tube, it seems reasonable to assume that mild inactive infections may not cause an increase sufficient to be recognized. An infection that is inactive in this regard need not be mild clinically. So, a relatively weak Widal may be found in a severe case of typhoid fever.

The following results have been obtained by various writers in cases where the diagnosis was definite:

*Primary Syphilis.*—Of 974 published cases, 56.5 per cent. reacted positively.[1] Various individuals have obtained from 38 to 90 per cent. of positives, most of them reporting from 50 to 75 per cent. The reaction usually appears about six weeks after infection. It has been reported as early as eight days after infection, which in this case was over eight days before the appearance of the primary induration.

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[1] These figures are abstracted from *Boas:* loc. cit.

*Secondary Syphilis.*—Of 2,762 reported cases, 88 per cent. were positive, various reports ranging from 77 to 100 per cent. In absolutely untreated cases the results are about 100 per cent. positive.

*Tertiary Syphilis.*—Of 830 reported cases, 80 per cent. were positive, different workers recording from 45 to 100 per cent. Practically all cases which have never been treated react positively.

*Latent Syphilis.*—Of 1,111 cases tested during the first three to four years after infection, 46 per cent. were positive. Of 1,452 cases of longer standing, 30 per cent. were positive. It is by no means uncommon for a patient to develop tertiary lesions after giving a negative reaction.

*Tabes Dorsalis.*—Of 360 sera, 70 per cent. were positive. Of 146 specimens of spinal fluid, 41 per cent. were positive in 0.2 c.c., but larger doses gave a higher percentage (page 698).

*Dementia Paralytica.*—Most writers report 95 to 100 per cent. of positives in tests with serum. Reports on spinal fluid vary from 52 to 90 per cent., depending largely on the amount used. Quantitatively the reactions are the strongest of those found in any condition except possibly hereditary lues.

*Congenital Syphilis.*—The statistics in this group of cases are unsatisfactory. The majority of infants with symptoms give a very strong positive. Many which give a negative reaction at birth give a positive about four weeks later and show symptoms. Some which give a positive at birth give a negative reaction shortly after (possibly a transfer of antibodies from the mother without infection). So that neither a positive nor a negative reaction is of definite prognostic value.

Mothers of syphilitic infants react in about the same percentage as patients with latent syphilis, and the percentage of reactions in those who have never shown symptoms is about the same as in those with a definite history of lues.

### NON-SYPHILITIC CONDITIONS IN WHICH REACTIONS HAVE BEEN OBTAINED

Some cases of scarlet fever, frambœsia, leprosy (especially the tubercular form), trypanosomiasis, and possibly malaria may give positive reactions. Blood from cadavers and from moribund patients may also react positively when syphilis can be excluded.

So far these are the only conditions which destroy the significance of a positive reaction. Aside from these groups, positives have been reported from time to time in an immense number of conditions, but the reports lack confirmation and many have been vigorously combated. There are two possibilities which may explain them: 1, that the individuals had also an unrecognized latent syphilis; and, 2, that a modified technique was used which made the reaction unduly sensitive. It is noteworthy that many of the reports have come from one laboratory. Among the conditions mentioned are typhoid fever, pneumonia, pulmonary tuberculosis, malignant tumors, cerebral tumors, lupus, Hodgkin's disease, myelogenous leukemia, diabetes mellitus, icterus, eclampsia, dementia præcox, multiple sclerosis, epilepsy, and Vincent's angina, and blood taken during narcosis. Positive results are also reported in pellagra and beri-beri, but have not been confirmed.

### INFLUENCE OF TREATMENT

A positive reaction may usually be made to disappear under specific treatment. This is accomplished most readily with mercury, less easily with iodides or arsenic. The effect seems due to the action of the drug in the body, and not to its presence in the serum when tested or to any direct action on the syphilitic antibody. The disappearance usually parallels the improvement in symptoms, but may precede them or may be indefinitely delayed. A serum which was negative before treatment may react positively after it. Much interest has attached to cases treated with salvarsan. After treatment with this drug the reaction may clear up immediately; it may persist for some time and then disappear; it may become negative and later again positive; or it may become positive when it was negative before injection. The prognostic significance in this connection is not known. Reactions in tertiary and parasyphilitic conditions are stronger and more frequent in untreated or poorly treated cases.

### VALUE OF THE TEST

In conclusion it may be stated that a positive Wassermann reaction (if certain conditions are excluded) is of great diagnostic significance, while a negative result is of very little value—it certainly does not warrant a favorable prognosis. It promises to be of great use clinically in differential diagnosis between gummata and tumors and in obscure tertiary lesions generally, and also in the diagnosis of tabes and general paresis. It gives evidence as to the syphilitic basis of many cases of arteriosclerosis and aortic insufficiency and chronic inflammations of the eye and ear of obscure etiology. A positive reaction in the primary stage may allow treatment to be begun before the exanthem appears, and a positive reaction in the latent stage seems to indicate that treatment has been insufficient (possibly not after salvarsan). It may be used as a guide to treatment especially if performed quantitatively from time to time.

It is possible that the reaction may prove of value in the selection and control of wet nurses and in the regulation of prostitutes, and finally in determining the expectation of life in examining for life insurance, inasmuch as statistics have been gathered showing that one-third of all persons suffering from syphilis finally succumb to some stage of the disease.

It is not possible to say that the reaction has any relation to the contagiousness of the disease, so that permission for marriage should not be based on a positive or negative finding, but rather upon the clinical condition.

## XII. DETECTION OF ARSENIC IN THE URINE

The general employment of dioxydiamidoarsenobenzol (salvarsan) and atoxyl in the treatment of syphilis has led to a considerable interest in the determination of the absorption of the drug by estimating the time of appearance and the amount of arsenic in the urine. After intravenous injections, the arsenic appears in the urine within a very short time, and excretion takes place very rapidly, so that the body quickly rids itself of the drug. After intramuscular injections, the arsenic may not appear for one or two hours and can be detected in the urine for weeks.

Only the qualitative method for its determination will be given here, as the quantitative method is contained in standard textbooks on quantitative analysis and is too complicated for general use.

The urine to be tested should be evaporated in a porcelain dish to about 100 c.c. The concentrated urine is then transferred to a smaller evaporating dish and about 10 c.c. of arsenic-free sulphuric acid added. The dish is heated over a free flame until the vapors of sulphuric acid are given off and the organic matter is reduced to a black mass. This procedure removes the nitrates and nitrites which would interfere with the further tests. The black residue is then extracted with 100 to 200 c.c. of boiling water and submitted to the Marsh test.

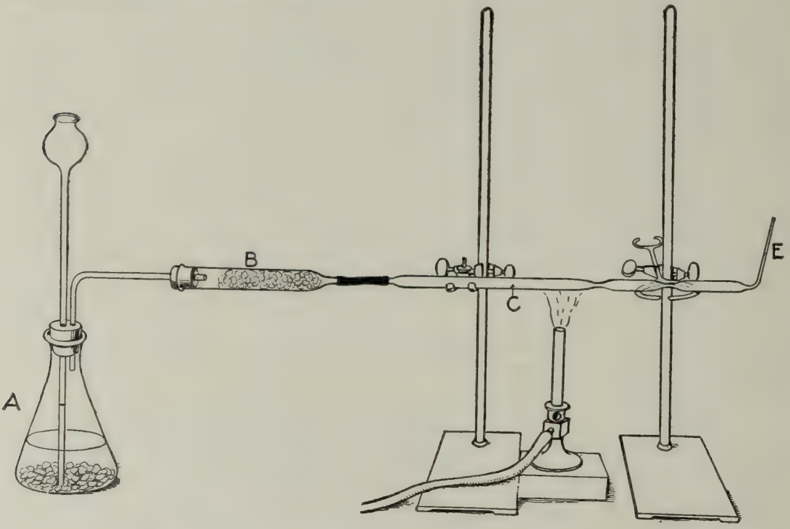

Fig. 181.—Marsh Apparatus for Arsenic.

This test depends upon the property of gaseous arsenic hydride ($AsH_3$) on being heated to break up into hydrogen and arsenic, the latter being deposited as a velvety black layer on the cool portion of the glass in which the heating takes place. Figure 181 shows the apparatus ordinarily used; (*a*) is the flask for evolution of $AsH_3$ by the action of sulphuric acid on zinc in the presence of arsenic; (*b*) is the tube filled with dried calcium chloride to dry the gas, and (*c*) is a tube of *hard* glass in which the $AsH_3$ is heated and decomposed. The flask should hold about 400 or 500 c.c. The zinc should be in granules and free from arsenic. The zinc should be covered with an approximately 3 per cent. solution of chemically pure copper sulphate for three to five minutes and then washed in distilled water. A granular coating of copper is thus deposited upon the zinc, which renders the evolution of gas more regular. About 200 c.c. of sulphuric acid diluted with eight volumes of water are poured into the flask. After the apparatus has run for about fifteen minutes a dry test tube is held over the upturned end (*e*) of the glass tube. After a few moments it is removed, closed with the thumb, and open end downward is held over a gas flame at a distance from the apparatus. If there is no explosion it shows that all the air has been displaced. The gas can then be lighted at the pointed end of the tube and the horizontal portion of hard glass heated with a Bunsen burner. The heating should continue for fifteen minutes. If at the end of this time there is no separation of arsenic the reagent can be assumed to be pure. A portion of urine treated as above is then poured into the apparatus. In order that no air may be introduced with the urine it is necessary to force the diluted acid up into the thistle bulb by compressing the rubber connecting tube; the fluid to be tested may then be poured on. On releasing the compression the combined fluids will enter the flask without carrying any air with them. The whole length of the hard glass tube should be thoroughly heated in order to drive out any moisture or volatile substance; then the whole flame of a Bunsen burner is placed under one of the expansions on the tube. If any arsenic is present the heat decomposes the arseniureted hydrogen with the production of a blackish mirror of metallic arsenic. When only traces of arsenic are present in the fluid tested, this mirror may be brownish in tone, but even very small quantities will usually give a black mirror. If any black deposit is produced in the tube beyond the Bunsen burner, the tube should be cooled and the spot tested to differentiate between arsenic and antimony. Arsenic dissolves immediately in a solution of sodium hypochlorite, while antimony dissolves very slowly, and on treatment with ammonium sulphide both dissolve. On evaporation of the solutions, the residue is yellow if arsenic be present, and orange if antimony. If a sufficient number of mirrors is available, one should be heated over a small flame, both ends of the tube being open to allow a current of air to flow through, and the white sublimate formed examined for the crystals of arsenic tri-oxide ($As_2O_3$). It is also well to make a blank test with solutions containing arsenic and antimony in case there is any doubt.

In testing for arsenic in the urine it is important to remember that traces of this substance may appear in the excreta following the ingestion of beer, syrups, or lemonade in the manufacture of which glucose has been employed. The arsenic is derived from the sulphuric acid used in inverting the starch. Traces of arsenic are also found in the urine of persons living in an atmosphere contaminated with soft coal smoke, in which there is usually enough vapor from arsenical pyrites to give a positive test in the urine. In all probability arsenic is not a regular constituent of the tissues, as has been stated by Gautier, but when found is due to intake in the food, to the use of spring water containing traces of arsenic, or to previous medication.

After taking a few drops of Fowler's solution, which contains about 3 mg. of $AsH_3$, arsenic can be obtained in the urine in about two hours; at the end of twenty-four hours, about one-third has been eliminated, but traces may still be found for four or five days. In applying the test, therefore, only frankly positive reactions should be considered.

## XIII. HÆMOLYTIC TESTS

Serious and even fatal results have been noted after direct transfusion of blood for therapeutic purposes. The symptoms point to an hæmolysis of the blood in the vessels of the recipient. It is generally assumed that this hæmolysis is due to the action of the serum of the patient upon the blood corpuscles of the healthy individual acting as donor, and hence it is considered advisable always to test the blood corpuscles of the donor against the serum of the recipient, in order to determine whether there is any hæmolysis *in vitro,* and to avoid transfusion in case such hæmolysis occurs, even though cases have been reported in which successful transfusion was done when slight laking of the corpuscles was observed in the test-tube experiment. It is much better, however, to test, not only the corpuscles of the donor against the serum of the recipient, but also the serum of the donor against the corpuscles of the recipient, as it is not impossible that hæmolysis may occur from the introduction of foreign serum into the vessels.

To do the test it is necessary to have a series of small test tubes, 8 or 9 mm. in diameter, and 10 cm. in length, some sterile physiological salt solution of a strength of 0.85 per cent., and some centrifuge tubes and several small containers for the clotted blood. These may be either homeopathic bottles or Wright capsules as shown in Fig. 178, No. V.

It is easiest to obtain the blood in a sterile syringe directly from a vein. If this is impossible, sufficient blood can usually be obtained by a deep puncture with a spear-pointed needle in the palmar surface of the finger. About 0.5 c.c. of blood from each person should be allowed to run directly into physiological salt solution in the centrifuge tubes. The rest of the blood is allowed to clot and the serum when expressed is collected with a sterile pipette. The corpuscles are centrifugalized and washed several times with saline solution in order to remove all adherent serum. The serum is collected in a sterile pipette, and measured quantities are placed in small test tubes, and the corpuscles are added up to one-tenth to one-fifth of the serum volume. The combinations are placed in the incubator for one hour to two hours. Mixtures should be made of the patient's serum and the donor's corpuscles, and of the donor's serum and the patient's corpuscles. Hæmolysis is shown by the appearance of a reddish color in the serum or, when marked, by the complete solution of the corpuscles.

It is often very convenient, especially if the quantity of blood is very small, to carry out these tests in the Wright capillary pipettes (Fig. 178, No. I), the corpuscles and the serum being drawn up in various proportions, the tip sealed, the pipette stuck upright in a glass of moist sand and placed in the incubator for one or two hours. When using these small quantities of blood, however, there is danger that a very faint hæmolysis may be overlooked, so that whenever possible it is well to use at least 1 c.c. of the patient's serum against the corpuscles of the donor, reserving a smaller quantity, if necessary, for the test between the donor's serum and the recipient's corpuscles. Even when there is but a slight degree of hæmolysis in the test tube, transfusion has frequently been followed by severe blood destruction and hæmoglobinuria. In some cases death has resulted from the introduction of relatively small amounts of blood. Any hæmolysis *in vitro* should be regarded as an absolute contraindication to transfusion.

A similar test has been used in determining the hæmolytic power of the blood of patients suffering from carcinoma. Such hæmolytic power is present in the serum of about 50 per cent. of such persons. Here, however, the serum must be tested against a considerable number of blood specimens derived from healthy persons as such serum may lake one blood and not another. As yet this reaction has but little diagnostic value.

Perhaps even more dangerous than hæmolysis is the intravascular agglutination of red cells; and convulsions and hæmorrhage, sometimes followed by death, have been observed after the injection of agglutinable blood. The agglutinins are determined in the same way as the hæmolysins. If these bodies are present, the cells will be found in a solid cake at the bottom of the test tube or, when the phenomenon is less marked, in granular clumps adherent to the side of the tube. As Ottenberg[1] has pointed out, agglutinins are of more import in the patient's blood than in the donor's, as their action is inhibited by the presence of an excess of cells. Consequently, it is of advantage in testing for these bodies, to use a 5 per cent. suspension of red cells instead of concentrated sediment.

The serum of one individual may also contain other bodies which induce phagocytosis of blood cells of another individual. These hæmopsonins or erythro-opsonins may be detected by the technique used in determining the opsonic index, using a 5 per cent. suspension of red cells instead of the bacterial suspension. Fatal symptoms have been observed after transfusion in the presence of those bodies.[2]

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[1] *Ottenberg:* Jour. Exper. Med., 1911, xiii, p. 425.

[2] *Hopkins:* Arch. Int. Med., 1910, vi, p. 270.

# PART IX

# MILK

## I. GENERAL CONSIDERATIONS

The mammary glands of infants of both sexes secrete a small quantity of milk for a short time following birth, and rarely a moderate secretion of milk has been noted in non-pregnant females and in adult males; but, aside from these few exceptions, the secretion of milk is a function of the female after delivery.

During the course of a pregnancy a small amount of a thin, yellowish fluid can be squeezed from the breasts, and the first milk after delivery has much the same physical characteristics. Chemically and microscopically, this colostrum, as it is termed, is distinguished from milk by the presence of a large amount of fat and salts, together with a small amount of sugar and of an albumin coagulable by heat. Numerous large cells from the walls of the alveoli and in a state of fatty degeneration are present. These cells are known as colostrum corpuscles. A few neutrophile leucocytes may also be found.

Normal human milk contains about 87 per cent. of water, 4 per cent. of fat, 2.5 to 3 per cent. of proteids and casein, 6 per cent. of lactose, and a small quantity of ash, usually less than 1 per cent. The fat is the most variable factor, and is the one most easily affected by disease. The color is a pale blue, the reaction faintly alkaline, the specific gravity between 1.028 and 1.032. Human milk is remarkable as compared to cow's milk, in its high proportion of sugar and low proportion of casein and ash. The casein differs markedly from that of cow's milk in not forming a distinct clot with acids, as does the latter, and in being coagulated with difficulty by chymosin. The curd from human milk is much finer than that from cow's milk. The casein probably differs chemically from the casein of cow's milk. The proteids are a lactalbumin and globulin.

Milk is rarely sterile, but usually contains bacteria derived from the lacteal ducts and the surface of the nipple. Microscopically nothing is to be seen, as a rule, but large numbers of fat globules arranged in small masses.[1] The composition of milk varies during the puerperium. The sugar increases rapidly after the birth of the child, while the fat increases slowly for several months. The casein and albumin are very high just after delivery, reaching nearly 9 per cent. By the seventh day they fall to 3.5 per cent., and at the end of the second week to 2.3 per cent. At the end of six or eight months the milk begins to diminish in quantity and to contain less fat, though the amount of solids is increased. Of the chemical changes which take place in the milk during disease but little is known, except in those conditions which cause a general reduction in the nutrition of the child and a corresponding change in the nutritive value of the milk, chiefly in the fat component.

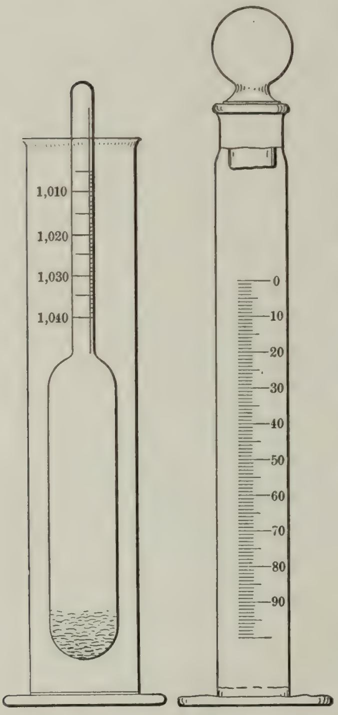

Fig. 182.—Holt's Milk-Testing Apparatus.

## II. CHEMICAL EXAMINATION OF MILK

The simplest method of testing milk is by the specific gravity and the cream tests. A small set of instruments for this purpose is on the market and is known as Holt's milk-testing set. It consists of two small graduated and stoppered tubes, in which the milk is to be placed and allowed to stand for twenty-four hours, when the amount of cream, and by this the fat, may be read off. The normal is 7 per cent. of cream by bulk equal to 4.2 per cent. of fat.

The other portion of the apparatus consists of a small cylinder and hydrometer float. Normal milk, if very rich in fats, will have a specific gravity approaching 1.030, and milk containing less than normal fats and other substances will also be lighter than very rich milk, and approach 1.028 as a lower limit, so that a diminution in specific gravity of milk may mean an increase in the fats or a diminution in the total solids. Which of these conditions is present will be indicated by the result of the cream test.

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[1] *Cohn:* Zur Morphologie der Milch, Virchow's Archiv, Bd. clxii, 1900, p. 187.

### REACTION

Normal milk is amphoteric in reaction to litmus paper; that is, it turns red litmus paper blue and blue litmus paper red. This is due to the presence of the phosphates of the alkalies with both acid and alkaline reaction. The milk, however, rapidly becomes acid, and the determination of this acid is of importance, as no milk should be Pasteurized which shows twenty-five degrees of acidity. This acidity is due to lactic acid formed in the milk by bacterial action, and such milk is not suitable food for young children. An acidity of twenty-five degrees does not perceptibly alter the taste or odor of the milk.

To determine the amount of acidity the milk should be titrated with decinormal sodium hydroxide,[1] using phenolphthalein as an indicator. Ten c.c. of the milk should be measured into a small beaker and diluted with 50 c.c. of water after the addition of two or three drops of a 1 per cent. solution of phenolphthalein in alcohol. The acidity may be expressed in degrees by considering each tenth of a cubic centimeter of sodium hydroxide required to neutralize 10 c.c. of milk as one degree. An acidity of fifteen to twenty-five degrees may be observed within six hours after milking, and forty-eight hours after the acidity may be as high as one hundred degrees.

### SPECIFIC GRAVITY

The specific gravity of milk may be determined either with a special lactometer, or with one of the ordinary urinometers used in determining the specific gravity of urine. The milk is put into a small cylinder and the instrument is lowered into it with a slight spin so as to avoid sticking to the side of the cylinder. When it has come to rest the graduation on the neck is read at the surface of the milk. The temperature of the milk should be 60° F., and the readings should be reduced by a table to give the correct specific gravity.

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[1] Lime-water is approximately one-twentieth normal, and may be used instead of $^{N}/_{10}$ sodium hydroxide.

A rough correction can be made by adding .0001 to the reading for each degree above 60° F., and subtracting the same amount for each degree below. For example, if the reading at 64° F. is 1.0285, the reading at 60° should be 1.0289.

The average density of cow's milk is 1.029. If the specific gravity is less than this it may be assumed that the milk contains either an unusual amount of cream, which may be decided by the Babcock test, or that the milk has been watered. If the percentage of fat is low and the specific gravity is also low, it is safe to assume that water has been added. If the milk tested is heavier than 1.029 and the percentage of fat is low, it may be assumed that the milk has been skimmed.

## TOTAL SOLIDS

The total solids of the milk can be determined from the lactometer readings by the following formula suggested by Babcock:

L = last two figures of the specific gravity corrected for temperature.

F = the percentage of fat contained in the milk.

$$\text{Solids not fat} = \frac{L}{4} + 0.2\ F.$$

$$\text{Total solids} = \frac{L}{4} + 0.2\ F \text{ plus } F.$$

Another method of determining total solids is by the use of the slide rule, such as has been suggested by Richmond. The instrument with full directions can be obtained from dealers in dairy supplies.

More accurate determinations require the evaporation of a measured bulk of milk. The residue is heated at 100° C. until its weight is constant.

## FAT DETERMINATION

The specimen of milk on which fat determination is to be made should be obtained with certain precautions. The mixing of the fat which has risen to the top of the bottle or the can should be very thoroughly carried out, as otherwise the results are not accurate. The best method is to pour the milk from the bottle into a large beaker and repeat this process a number of times. Then the necessary quantity should be promptly removed by a pipette. Milk in large cans should be poured out of the can into another one and this repeated several times. The fat rises very rapidly to the top and in ten or fifteen minutes a considerable separation has taken place, so that the upper and lower layers of the fluid give widely distant results.

**Adam's Method.**—The most accurate procedure for determining the fat is as follows: A strip of fat-free absorbent paper, twenty-two inches long and two and one-half inches wide, is rolled into a loose coil and fastened by twisting a fine piece of copper or platinum wire about it. Five c.c. of milk is then carefully run on to the upper end of the coil of paper. The coil is then dried at a temperature of about 100° C. for an hour. When dry, the wire should be removed and the coil placed in a Soxhlet apparatus and the fat extracted with gasoline.

The fat will be removed after the apparatus has been running for about two hours. The flask is then removed, the gasoline is evaporated at a low temperature, and the fat determined by weight. Ether may be used instead of gasoline, but it must be perfectly anhydrous,[1] otherwise the milk sugar will be extracted and increase the weight of the fat.

**Babcock Method of Fat-Testing.**—The method depends upon the destruction of the proteid of the milk by means of strong sulphuric acid and the separation of the melted fat by centrifuging the mixture. If a considerable quantity of milk can be obtained the most accurate results are procured by using the standard commercial bottles (Fig. 183), which hold about 17.6 c.c. of milk and about an equal

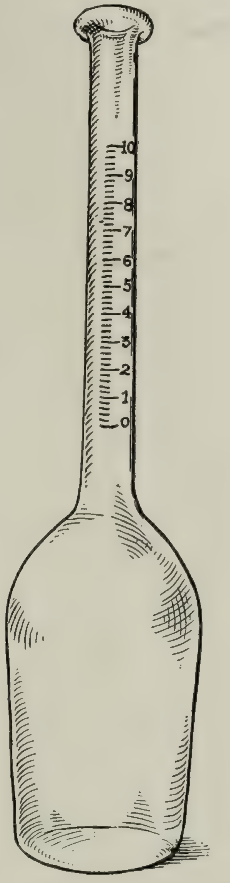

FIG. 183.—BABCOCK MILK TUBE.

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[1] See Appendix, p. 734.

quantity of acid. The milk should be measured out in a pipette which holds, when filled to the mark on the stem, 17.6 c.c. After filling the pipette the test bottle is filled by inclining it slightly and placing the point of the pipette in the neck of the bottle; the milk is thus allowed to run slowly down the side of the neck, thus providing an exit for the air in the bottle. The pipette is allowed to drain for about half a minute and then the last drop is blown into the bottle. After the milk has been measured into the bottle the acid should be added. This may be done at once, or the milk may be allowed to stand in the test bottle for a number of days without changing the result. The acid pipette or graduate should contain about 17.5 c.c., and should be filled with sulphuric acid of a specific gravity of between 1.82 and 1.83 at 20° C. The bottle should be inclined and the acid poured down the neck so as not to drop into the milk but flow down the side and thus avoid charring the milk. The two layers should now be mixed by a combination of a rotary and shaking motion, special care being taken not to allow any curds to get into the neck of the bottle. The shaking should be continued until all particles of the curd are dissolved; the liquid will then be of a dark brown color and very hot, owing to the chemical action of the acid on the milk. The test bottles are now placed in a centrifugal machine and whirled for five minutes. It is well to fill the shields of the centrifuge with boiling hot water in order to keep the milk fat melted during the process of centrifugation.

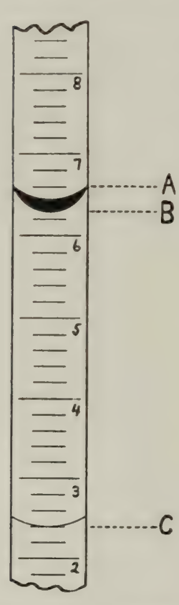

Fig. 184.—Diagram to show Method of Reading Babcock Tube. The reading is from A to C, not B to C. (Wing.)

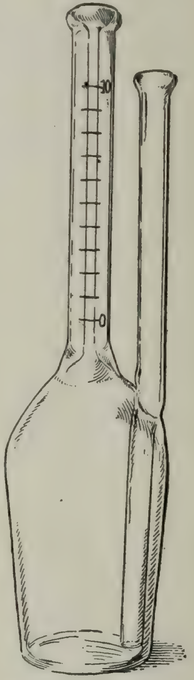

Fig. 185.—Babcock Bottle for Skimmed Milk.

After about five minutes the machine is stopped and boiling hot water is added to the bottles in order to bring the fat up within the graduated portion of the neck, and the centrifugalization continued for one minute. If the bottles have been kept at the proper temperature the fat will be liquid and clear, with a sharply formed meniscus, and below it will be clear water. To read off the amount, a pair of dividers are placed with their points at the bottom of the lower meniscus and at the upper limit of the fat (see Fig. 184). The dividers are then placed at the zero of the scale and the amount of space covered by the fat is read off. Each space of the neck of the bottle represents two-tenths of one per cent. of fat. The larger spaces, numbered 1, 2, 3, etc., each represent one per cent. In testing skim milk special bottles should be used giving readings to one-twentieth of one per cent. (Fig. 185). The pipette and acid measure are used and the fat is estimated in the same way as for full milk, except that it is sometimes desirable to use a little more acid.

For testing cream, bottles should be used having a wide neck which read up to 30 per cent. of fat, and 18 c.c. of cream should be used instead of 17.6 c.c., as cream is lighter than milk. In testing thick cream it is better to weigh out 18 grams on a balance, because of the difficulty of pouring the thick cream from the bottles and the irregular masses of fat. After the test has been completed the bottles should be immediately rinsed with hot water, and, if necessary, a little caustic soda solution to remove traces of fat. This should be done before the fat in the neck of the bottle has time to harden. When small quantities of milk only can be obtained, as in testing breast milk, smaller tubes must be used which require only 5 c.c. or even smaller amounts.[1]

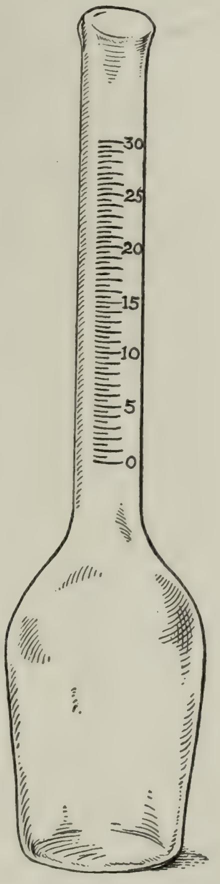

FIG. 186. — BABCOCK TUBE FOR CREAM.

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[1] See *Lewi:* Archives of Pediatrics, vol. xv, 1898, p. 179.

Lewi has devised a bottle for this purpose which requires 2.92 c.c. of milk. That amount of milk is measured off in the small pipette which is provided with the apparatus and mixed in the bottle with 3 c.c. $H_2SO_4$. Six-tenths c.c. of a mixture of equal parts of concentrated hydrochloric acid and amyl alcohol is then added and the bottle is then filled up to near the top with equal parts of sulphuric acid and water. It is placed in a special aluminium shield which is sold with the outfit and which is of a suitable size to enter the collar of the ordinary medical centrifuge, and the specimens are centrifuged for two minutes at about one thousand revolutions per minute. The addition of the amyl alcohol aids in the separation of the fat and gives clearer readings than with sulphuric acid alone. The mixture keeps for several weeks, but when it turns dark is unfit for use and should be freshly prepared. Each tenth of a division on the graduated neck of the bottle is equivalent to 0.3 per cent. of fat, so that if the reading be one and one-tenth division, the amount of fat present in the milk is three and three-tenths per cent.

It is well to make two tests and average the results, as errors are likely to be introduced owing to the small amount of milk used and the difficulties connected with the reading of the column of fat.

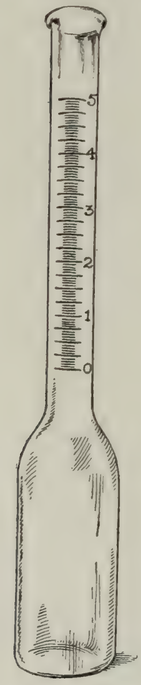

Fig. 187. — Bottle for Babcock Test to fit Medical Centrifuge. (Lewi.)

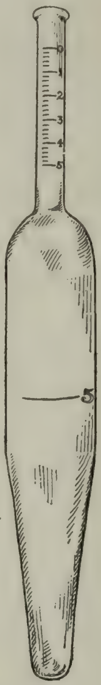

Fig. 188.—Small Babcock Tube for Medical Centrifuge.

Another form of bottle is made to fit the conical shields of the centrifuge. It requires 5 c.c. of milk, an equal quantity of sulphuric acid, specific gravity 1.83, and enough of the amyl alcohol-hydrochloric acid mixture to fill the tube. The divisions read to two-tenths per cent. The narrow neck of these bottles renders them very difficult to fill and clean, otherwise they are fairly satisfactory.[1] (Fig. 188.)

### ESTIMATION OF THE SUGAR

The casein is precipitated by acidulating the milk with acetic acid, and the albumin by boiling the acidulated mixture. The coagulum is filtered off, and the filtrate after cooling is placed in a burette and titrated with Fehling's solution or with Pavy's modification, as is described under Urine. It is to be remembered that the lactose in the milk does not reduce the copper solution as rapidly as does glucose, so that the boiling of the fluid must be continued for some time longer than is the case with the latter form of sugar. The reduction factor for lactose is also different from that for glucose, and instead of 10 c.c. of Fehling's being equivalent to 0.05 gram, it is equivalent to 0.06 gram of lactose.

### ESTIMATION OF THE PROTEIDS IN MILK

Woodward has devised a simple method which is accurate enough for clinical purposes. The milk is placed in two small separatory funnels or milk burettes holding 5 c.c. each and allowed to remain in the incubator for eighteen to twenty-four hours. At the end of this time the fat of the milk will have risen to the top in the form of a thick, viscid layer, leaving the proteid and the sugar in solution below. The tubes are allowed to cool and the milk is drawn off into small Esbach tubes, a sufficient quantity of the reagent added, and the tubes centrifuged for a few minutes until the height of the precipitate remains constant. The amount of albumin may then be read off from the graduations on the tube in parts per thousand. It is evident that the method is only approximate, as the errors of the Esbach method are very considerable.

The proteid in cow's milk can not be estimated in this manner because of the coarse coagulum which is produced by the casein.

Another method of estimating the total proteids of both human and cow's milk is by determining the nitrogen content of a measured quantity by the Kjeldahl method. As human milk contains about 9 per cent. of non-proteid nitrogen and cow's milk about

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[1] For a discussion of the errors of the various methods just described, see *Shaw:* Trans. Am. Pediatric Soc., vol. xv, 1903, p. 76.

6 per cent., the quantity of nitrogen obtained by the Kjeldahl process should be diminished by this amount and then multiplied by 6.34 to convert it into its equivalent of proteid.

Another method which may be used for the separate determination of casein and albumin in cow's milk is as follows:[1]

**Casein.**—Ten c.c. of the milk sample are diluted with about 90 c.c. of water at a temperature of 40° C. and 15 c.c. of a solution containing 10 per cent. by weight of acetic acid. The whole is allowed to stand for five minutes, washed three times by decantation, pouring the washings through the filter, and the precipitate is finally transferred completely to the filter. If the filtrate is not clear at first it may be returned through the filter several times. The filter and its contents are then oxidized by the Kjeldahl method. The nitrogen multiplied by 6.38 gives the casein.

**The albumin** may be determined in the filtrate by neutralizing a solution of sodium hydroxide, adding a trace of dilute acetic acid, and heating the mixture fifteen minutes. The coagulum is collected on the filter, washed free from salts, and the nitrogen determined. The amount of nitrogen obtained multiplied by 6.25 gives the albumin.

## TESTS FOR PRESERVATIVES IN MILK

It may be convenient at times to be able to test commercial milk for certain adulterations by chemicals added to the milk with a view to its better preservation, so that tests for the three chief substances used are given below.

**Tests for Formaldehyde.**—1. Add to the suspected milk some strong hydrochloric acid and a few drops of a 10 per cent. solution of ferric chloride; if formaldehyde is present a violet color will appear on gently heating the mixture.

2. Place 2 c.c. of strong sulphuric acid in a test tube and add a trace of ferric chloride; then pour on the surface of the acid mixture a few cubic centimeters of the suspected milk in such a way that the two fluids are not mingled but remain in layers. A violet color at the point of juncture is an evidence of the presence of formaldehyde.

3. Add 10 c.c. of milk to 1 c.c. of decolorized fuchsin solution,

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[1] *Leffman* and *Beam:* Food Analysis, Phila., 1901.

shake and allow to stand for five minutes, then add 2 c.c. of hydrochloric acid. A violet color indicates formaldehyde.

The decolorized fuchsin solution is made by passing gaseous sulphurous acid into a moderately strong aqueous solution of fuchsin until the red color disappears. The sulphurous acid can be easily generated by treating a solution of commercial sodium bisulphite with sulphuric acid diluted with an equal bulk of water. The acid should drop slowly into the bisulphite solution, and the gas evolved is conducted by a tube into the fuchsin solution.

4. Two c.c. of milk and an equal amount of water are mixed in a test tube and 1 decigram of phenylhydrazin hydrochloride is added and 10 c.c. of 10 per cent. NaOH. The mixture is shaken for a minute. If formalin is present the whole will assume a rose-red color.

It is important to remember that all these reactions are in no sense a test for formaldehyde; they are merely the general reactions for the aldehyde group, of which formaldehyde is the only member used in the preservation of milk. There is no simple test for formaldehyde in small quantities.

**Salicylic Acid.**—The use of salicylic acid is becoming less frequent, especially since the acid imparts a bitter sweetish taste to the milk, and it is being replaced by formaldehyde.

To test for the acid, the proteids must first be precipitated by the addition of a sufficient quantity of mercuric nitrate. A solution of the latter is made up by dissolving mercury in twice its weight in nitric acid and diluting the whole five times. The liquid is then filtered and shaken out with about half its volume of a mixture of equal parts of ether and petroleum ether. The ethereal solution is evaporated and a few drops of ferric chloride are added to the residue. A violet color indicates that salicylic acid is present.

**Boric acid and the borates** are used extensively to adulterate milk. Ten c.c. of the milk are evaporated and the residue incinerated. The ash is then moistened with very dilute HCl, just sufficient to render the solution acid. A slip of turmeric paper is then placed in the fluid, which is evaporated over a water-bath. If boron compounds are present, the moistened portion of the turmeric will change to a brownish-red color.

An easier plan is to evaporate and incinerate as before, and then to moisten the residue and, taking up a little of the latter on a platinum wire, volatilize the boron in a Bunsen flame. The green flame will show the five green bands characteristic of boron when examined with a spectroscope.

## III. BACTERIOLOGICAL EXAMINATION OF MILK

The practitioner, even with a limited amount of apparatus and a slight knowledge of bacteriological technique, can make examinations of milk which often offer results of considerable practical value. The problem is not to isolate pathogenic bacteria from the milk, but merely to determine the number of bacteria of all sorts which may be present. The results of such enumerations, if they are high, merely show that the sample of milk examined has been exposed to a large amount of contamination. Such milk may at any time contain pathogenic germs derived either from a cow, from the water used in washing the pails, or from the hands of the milkers. On the other hand, if a specimen of milk contains only a few bacteria, say from ten to thirty thousand to the cubic centimeter, one may be reasonably certain that considerable care has been used in the collection and preservation of the milk, and that contamination from either human or bovine sources is not probable.

The isolation of pathogenic bacteria directly connected with an epidemic of infectious disease has been but rarely accomplished.

**Technique.**—In order to enumerate the bacteria contained in a sample of milk, care should be taken to select a representative specimen. If the milk is in cans, one which has not been opened should be selected and the milk poured into another can which has been thoroughly cleansed. After the milk has been poured back and forth for several times, the cream, which often contains an extra allowance of bacteria, will have been thoroughly mixed with the milk, and a sample taken will be fairly representative of the number of bacteria to the cubic centimeter. One hundred c.c. should be taken if fat and other tests are also to be made. A convenient receptacle is a small sterilized Erlenmeyer flask plugged with cotton.

In order to enumerate the number of colonies of bacteria in the milk it is necessary to dilute it as follows:

A flask of nutrient bouillon is opened and 99 c.c. of its contents are removed by a sterile pipette and placed in a second sterile flask. One c.c. of the milk to be tested is then added, also by means of a sterile pipette. One c.c. of this mixture contains 0.01 c.c. of milk. A series of agar tubes should be liquefied and brought to a temperature of 43° C. One c.c. of the diluted milk is added to No. 1; 0.1 c.c. to No. 2; and 0.01 c.c. to No. 3. The dilution in No. 1 will be 1 to 100; in No. 2, 1 to 1,000; and in No. 3, 1 to 10,000. If further dilutions are necessary, 1 c.c. of the original milk-bouillon mixture should be transferred to a second 99 c.c. of bouillon, thus making a dilution of 1 to 10,000. One-tenth c.c. of this will give a dilution of 1 to 100,000, beyond which it is rarely necessary to go. Occasionally, however, for market milk, it is necessary to dilute 1 to 1,000,000. In other words, to take 0.01 c.c. of the 1 to 10,000 mixture.

The milk is thoroughly mixed with the contents of the agar tubes, and before the latter have time to solidify they are poured into Petri dishes, care being taken to allow as little as possible of the softened agar to reach the sides of the plate. As soon as the plates have solidified they are transferred to an incubator at 37° C. At the end of two days the colonies are counted. In plate No. 1, containing the 1 to 100 mixture, if ten colonies are present it is an evidence that 1,000 bacteria are contained in 1 c.c. Thirty colonies in a 1 to 10,000 mixture means that 300,000 bacteria are present in a cubic centimeter, etc.

It is convenient not to have more than 100 colonies to a plate, as otherwise the counting becomes a matter of considerable difficulty. A series of dilutions should therefore be made up, judging the dilutions according to the probable bacterial content of the milk. Some observers prefer to count the plates at the end of four or five days, allowing two days in the incubator and three days at room temperature. Much larger counts are obtained in this way, the bacterial increase at room temperature being chiefly due to the group of water bacteria.

The number of bacteria found in milk varies from a few thousand to 10,000,000 per c.c. The lower counts are seen only in the sealed and bottled milk from high-grade dairies, where care is taken to sterilize everything which comes in contact with the milk.

Good country milk obtained without any especial precautions runs from 50,000 to 100,000 within a day after milking, or often higher, while thirty-six hours after the number of bacteria may be very large.

In mixed milk from a single dealer as sold in cans by wholesale in New York, the writer has found from 50,000 to 3,000,000 bacteria, without much relation to the time of year. The cheaper grade of "grocery" milk, which is often forty-eight to seventy-two hours old, competes very favorably in its bacterial content with sewage. Counts of 10,000,000 are not infrequent.

# APPENDIX

## THE PREPARATION OF STAINING FLUIDS

As has already been stated on page 81, it is much more satisfactory to purchase certain stains already prepared from Grubler. This is especially true of the Ehrlich triacid and Jenner stains, and in general it is better to obtain all of the aniline dyes from the same source. Much more uniform results are possible than if the dyes are purchased without reference to the source from which they are procured. In this country, a number of the large houses dealing in scientific apparatus keep the Grübler dyes in stock, so that they may always be obtained without the delays incident to importation. Care should be taken that the containers are stamped with Grübler's name.

A number of the aniline dyes are conveniently kept in the form of saturated solutions. The following list gives the number of grams of dye in 100 c.c. of the solvent. The amounts are approximate only, for they vary with different makes of the same dye, and with the temperature at which the solution is made up. Supersaturated solutions do not always deposit the excess of the dye on cooling.

| | | |
|---|---|---|
| Fuchsin solution in 96 per cent. alcohol | 3.0 | grams |
| Gentian violet solution in 96 per cent. alcohol | 4.8 | " |
| Gentian violet solution in water | 1.5 | " |
| Methylene blue solution in water | 6.7 | " |
| Methylene blue solution in 96 per cent. alcohol | 2.0 | " |
| Scharlach R. solution in 70 per cent. alcohol | 3.2 | " |
| Sudan III solution in 70 per cent. alcohol | .2 | " |
| Thionin solution in 50 per cent alcohol | .6 | " |
| Thionin solution in water | 1.2 | " |

The formulæ for the Ehrlich triacid, the Jenner, Wright, and other stains which require a rather elaborate technique, have been given in full on pages 81–90, and need not be considered here. Those which follow have been collected for convenience of reference.

**Carbol-fuchsin.**—Ninety c.c. of a 5 per cent. aqueous solution of carbolic acid are mixed with 10 c.c. of a saturated alcoholic solution of fuchsin. The mixture keeps for a year or more.

**Gabbett's Decolorizing Mixture.**—Two grams of methylene blue are dissolved in 100 c.c. of 25 per cent. sulphuric acid (sp. gr. 1.18).

**Aniline Water-Gentian Violet.**—One c.c. of aniline oil is shaken up with 25 c.c. of distilled water and the emulsion filtered through a moistened filter paper, which retains the excess of oil. Eighteen c.c. of the fluid are mixed with 2 c.c. of a saturated solution of gentian violet in strong alcohol. The staining mixture should be filtered before using, and keeps only a few days.

**Carbol-thionin.**—Twenty c.c. of a saturated solution of thionin in 50 per cent. alcohol are added to 100 c.c. of 2 per cent. carbolic acid in water. This is an excellent stain for bacteria as well as for the malarial parasite.

**Loeffler's Methylene Blue.**—Thirty c.c. of a saturated solution of methylene blue in strong alcohol is mixed with 100 c.c. of a one to ten thousand aqueous solution of potassium hydrate.

**Weigert's Stain for Elastic Tissue.**—Two grams of resorcin and one gram of fuchsin (basic) are dissolved in 200 c.c. of water and the mixture heated to the boiling point. Twenty-five c.c. of liquor ferri sesquichloridi (Ph. Ger. III) are added and the boiling continued for five minutes. The mixture is cooled and the precipitate collected on a filter, dissolved in 200 c.c. of hot 95 per cent. alcohol, and, after cooling, again filtered. Four c.c. of strong hydrochloric acid are then added and the stain is ready for use.

**Orcein Stain (Unna-Tänzer).**—One gram of orcein is dissolved in 80 c.c. of strong alcohol and 35 c.c. of distilled water. After solution is completed, forty drops of strong hydrochloric acid are to be added.

**Gram's Solution of Iodine.**—One gram of iodine and two grams of potassium iodide are dissolved in 300 c.c. of water.

**Stains for Fat.**—Saturated solutions of Sudan III and scharlach R. should be made up in 70 per cent. alcohol. A small amount of alkali added to the solution acts as a mordant and gives a somewhat denser stain, but is not essential.

## APPARATUS

**Glass Utensils.**—Beakers and flasks, especially the flasks used in Kjeldahl nitrogen analyses, should be of Jena glass. Despite its relatively high cost, this form of glass withstands sudden changes in temperature better than any other variety on the market, and in the long run is more economical than the cheaper sorts. The best glassware of domestic manufacture is sold under the trade names of "Nonsol" and "Baloc."

Glass rods and tubing should be of hard sodium glass, not of soft lead glass, as the latter is easily dissolved by chemicals. "Durax" Jena glass should be used when platinum wires are to be sealed into the ends of tubes or rods. The coefficient of expansion of this glass is approximately that of platinum, and the glass withstands sudden variations in temperature better than ordinary varieties.

Pipettes of varying capacities are frequently used in medical chemistry, the most convenient form being that known as the outflow pipette. That is, the pipette is so calibrated that the desired quantity of fluid is delivered by allowing the pipette to empty itself and then blowing out the last drop. The ordinary commercial pipettes are, as a rule, not very accurate, and for special analytical work either certified pipettes should be obtained or the laboratory worker should calibrate his own by weighing the amount of distilled water delivered by the pipette. Almost all ordinary pipettes deliver the fluid which they contain much too rapidly to afford accurate results. The time required for a pipette to empty itself should be at least 40 seconds. The best way of obtaining this slowness of discharge is to heat the lower end of the pipette in a Bunsen flame until the glass is softened and contracts somewhat.

The accuracy with which the pipette delivers the fluid to be measured depends very largely upon its cleanliness. If the inner surface of the pipette is greasy, drops of water or other fluids will be retained in the bulb, and the quantity delivered may be too small by 1 or even 2 per cent. of the total bulk. The best means of thoroughly cleansing the pipettes is to allow them to stand in a tall jar containing a mixture of potassium bichromate and sulphuric acid (battery fluid[1]) for several days, then to wash with water and allow the pipette to drain on a piece of filter paper with the mouth end downward.

The proper form for accurate pipettes is shown in Fig. 189, *A*. The shape shown in Fig. 189, *B*, is incorrect, though such pipettes are convenient in obtaining small quantities of fluid from test tubes when accuracy is not important, and where the fluid handled is infectious or gives off strong fumes. The low position of the bulb enables the pipette to be filled without aspiration.

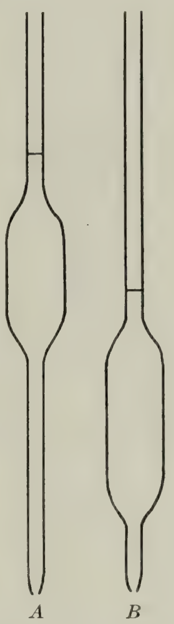

Fig. 189.—Correct and Incorrect Forms of Pipettes.

Pipettes for measuring small quantities of fluids are often arranged so that the whole content of the pipette is not allowed to run out, but the fluid is permitted to flow until a mark on the lower stem of the instrument is reached. The errors due to a larger or smaller drop remaining in the tip of the pipette are thus avoided.

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[1] Potassium bichromate or crude chromic acid, 250 grams; strong sulphuric acid, 300 c.c.; water, 2,000 c.c.

It is often convenient to have pipettes so arranged that a large rubber bulb can be fitted over the upper end. The delivery of small quantities of fluid can be more accurately accomplished than when the upper end is closed by the finger.

If the upper end of the pipette is *ground* smooth it is much easier to regulate the flow of the fluid, especially if the pipette is slowly rotated in the fingers to permit the entrance of a small amount of air, than if the upper end is simply rounded off in a flame, as is usually the case in the commercial article.

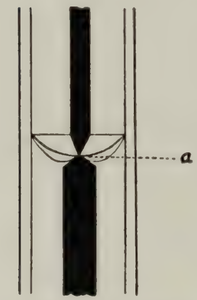

FIG. 190.—DIAGRAM TO SHOW METHOD OF READING BURETTE WITH A SCHELLBACH STRIPE. The mark *a* where the two points touch gives the height of the fluid.

Graduated flasks for making up standard solutions are conveniently of the Giles' type (see Fig. 192), and should be standardized to be used at a temperature of 20° C.

Burettes should be provided with glass stopcocks, and are preferably made with blue and white stripes down the side opposite the graduation, the so-called Schellbach graduation. This enables the height of the meniscus to be determined with great accuracy and avoids the use of a burette float. The diagram (Fig. 190) shows the method of reading the burette, the mark *a* showing the point at which the reading is to be made.

**Platinum Apparatus.**—Platinum crucibles require a good deal of care to prevent injuring the metal by alloying it with lead or other fusible metals. Compounds containing lead, arsenic, mercury, or antimony, should never be heated in a platinum crucible. Stains may be removed by burnishing the crucible with fine *sea sand* or by fusing potassium bisulphate in the crucible.

Platinum wire for loops for making smears or inoculating culture media should be of two sizes, No. 24 and No. 26 gauge. The heavier wire is preferable for making stab cultures or spreading pus or thick fluids on slides or cover glasses. The thinner wire cools much more readily after heating, and is a convenient size for carrying small quantities of culture material from one tube to another. Both varieties of wire should be hardened by alloying the platinum with small quantities of iridium.

The loops at the ends of wires should be made permanent by welding. In order to weld platinum it is only necessary to bring it to a white heat and then tap the two fragments of wire which are in contact with a small hammer or pinch the wires together by means of a pair of pliers. If the wire is clean and heated to the proper temperature, a firm union between the two portions forming the loop will be obtained and the excess of wire may be trimmed off with a file. Such loops are quite permanent, and are convenient because they keep their dimensions and form and do not have to be continually remade.

Spreads of thick fluids or scrapings from tuberculous lymph nodes are conveniently obtained by using a flat platinum spatula, which can be made by hammering out a short piece of No. 16 or No. 18 gauge hardened platinum wire.

Platinum loops and spatulæ are conveniently mounted in aluminium handles, but the usual custom is to fasten the wire into the end of a glass rod. The rod or tubing used for this purpose should be of the "Durax" Jena glass mentioned above or a variety designated $397^{\text{III}}$.

**Filter Paper.**—Ordinary cheap filtering paper may be used for the filtration of urine previous to carrying out the various tests for albumin. For chemical analyses it is much better to use Schleicher and Schüll's filter papers. The increased cost is compensated by the ease and rapidity of filtration and the completeness with which fine precipitates are retained A good paper for general use is Schleicher and Schüll's No. 597. For quantitative analyses Schleicher and Schüll's No. 589 should be used.

In making Jenner and Wright stains and in filtering large quantities of fluids, especially those which are alkaline, Schleicher and Schüll's hardened filter paper No. 575 should be employed, as it does not tear easily when wet, and its smooth surface allows a moist precipitate to be scraped off without removing any of the fiber of the paper. This paper can be used repeatedly.

## REAGENTS

The following list of reagents is sufficient to carry out most of the tests given in the text:

Alcohol, amyl.

Alcohol, ethyl, 96 per cent. and 80 per cent.

Alcohol, methyl, Merck's or Kahlbaum's pure.

Alkaline barium chloride solution. Composed of two volumes of saturated solution of barium hydrate and one volume of a 10 per cent. barium chloride solution.

Ammonium carbonate.

Ammonium chloride, 10 per cent. solution.

Ammonium hydrate, specific gravity 0.96. Contains about 10 per cent. of $NH_3$.

Ammonium molybdate.

Barium chloride, 10 per cent. solution.

Barfoed's reagent.—Thirteen and three-tenths grams of cupric acetate are dissolved in 200 c.c. of water, and 6 c.c. of 38 per cent. acetic acid are added.

Bromine and bromine water. The latter is prepared by mixing water with an excess of bromine, allowing the mixture to stand until the water has assumed a dark color. The supernatant fluid is then poured off.

Calcium chloride.

Chloroform.

Copper sulphate, 10 per cent. solution for general tests.

Copper sulphate, 20 per cent. solution for Hiss' capsule stain.

Copper sulphate, Fehling's solution. One liter of this solution contains 69.278 grams of crystalline copper sulphate.

Ether. Should not give a residue on evaporation. For fat extraction the ether should be dehydrated by first washing with water, then solid sodium hydroxide is added until most of the water is removed, and finally a few clean pieces of metallic sodium should be added, and the ether kept in a *loosely stoppered bottle.*

Ferric chloride, 10 per cent. solution.

Formalin.

Gasoline, specific gravity 86° Baumé. The boiling point should be near 50° C.

Hypobromite solution, 500 c.c. water, 200 grams NaOH, 12.5 c.c. bromine; another method is to take 70 c.c. of a 30 per cent. solution of sodium hydrate (NaOH), dilute with 180 c.c. of water, and add 5 c.c. of bromine.

Iodine.

Lead acetate, 10 per cent. solution.

Lead subacetate. Made by mixing three parts of lead acetate, one part of litharge, ten parts hot water.

Magnesium mixture. Composed of one part of crystallized magnesium sulphate, two parts ammonium chloride, four parts ammonium hydroxide, and eight parts water.

Phenylhydrazin.

Potassium chromate, 5 per cent. solution.

Potassium ferricyanide crystals. (The solution does not keep well.)

Potassium ferrocyanide, 10 per cent. solution.

Potassium hydrate, 30 per cent. solution.

Potassium iodide.

Potassium permanganate (Kahlbaum's pure). Used in titrating uric acid, etc.

Silver nitrate.

Sodium chloride. Saturated solution. One hundred c.c. of this solution contains about 31 grams of sodium chloride. Physiological salt solution contains 0.85 per cent NaCl.

Sodium hydrate, 30 per cent. solution.

Sodium nitrite.

Uranium nitrate solution containing 35.461 grams to the liter.

Zinc chloride solution. Prepared by mixing a thick, watery solution of zinc chloride with alcohol, until the specific gravity is about 1.20.

Esbach reagent. Ten grams picric acid, 20 grams citric acid, 1,000 c.c. of water.

Fehling's alkaline solution. Three hundred and forty-six grams of sodium and potassium tartrate (Rochelle salt) and 100 grams of sodium hydrate are dissolved in water, and the whole made up to 1,000 c.c. after the fluid has cooled.

Millon's reagent. One part of mercury is warmed with two parts of strong nitric acid, until it is fully dissolved. Two volumes of water are added to each volume of the solution of mercuric nitrate.

Nylander's solution. Twenty grams of bismuth subnitrate and 40 grams of Rochelle salt are dissolved by warming gently in a liter of 10 per cent. sodium hydrate.

Obermayer's reagent. Concentrated hydrochloric acid (sp. gr. 1.19) containing 2 grams of ferric chloride to the liter.

Robert's mixture. One volume of concentrated nitric acid and five volumes of a saturated aqueous solution of magnesium sulphate.

Spiegler's reagent. Eight grams of mercuric chloride, 4 grams of tartaric acid, and 20 grams of glycerin in 200 c.c. of water.

Acetic acid, glacial, 30 per cent. and 2 per cent.

Hydrochloric acid, strong.

Nitric acid. Strong, for albumin tests in urine. Twenty-five per cent. for decolorizing tubercle bacilli. Fuming for bile tests.

Sulphanilic acid for diazo-reaction.

Sulphuric acid. Concentrated, sp. gr. 1.84, for the Kjeldahl process. Sp. gr. 1.83, for the Babcock fat test; and 5 per cent. for decolorizing tubercle bacilli.

## INDICATORS

The indicators used in clinical chemistry are chiefly the following:

**Methyl Orange.**—Five centigrams of the dye are dissolved in 100 c.c. of distilled water.

**Di-methyl-amido-azobenzol.**—Half a gram of the dye is dissolved in 100 c.c. of 96 per cent. alcohol. Two drops of the solution are sufficient for titrating 20 or 30 c.c. of fluid.

**Congo Red.**—A saturated solution of the dye in distilled water is used by Boas for determining the free hydrochloric acid in the stomach contents.

Congo paper can be made by soaking strips of smooth filter paper in the solution and drying in the air.

**Alizarin** (*Alizarin S. or Alizarin sulphonate of soda*).—One gram of the dye is dissolved in 100 c.c. of water and filtered from the undissolved residue. The solution does not keep more than a few months. A few drops of the solution in water give a yellowish color, the shade changing to red by the addition of alkalies, and finally to a violet when an excess has been added.

**Litmus.**—This substance may be dissolved in strong alcohol and a few drops added to the solution to be tested, or it may be kept in aqueous solution, but only for a short time. It is better to dissolve a little of the powder when needed.

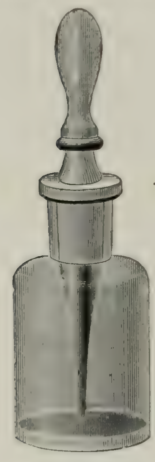

FIG. 191. — DROPPING BOTTLE FOR DYES OR INDICATORS.

Neutral litmus paper may be made by dissolving litmus in hot water and neutralizing the solution carefully with ammonia or oxalic acid, and then soaking strips of filter or letter paper in the fluid.

Acid litmus paper may be made by dripping filter or, what is better, letter paper, in a solution rendered faintly acid with oxalic or acetic acid. For alkaline paper the solution should be made faintly alkaline by ammonia or sodium hydrate.[1]

**Phenolphthalein.**—A 1 per cent. solution in either 50 or 96 per cent. alcohol is employed. One or two drops of this solution are sufficient to give a deep red color when a solution is rendered alkaline.

**Lacmoid.**—This is an indicator used in estimating the alkalinity of the blood. It may be used in solution or, preferably, in the form of lacmoid paper. This paper is prepared by soaking smooth-surfaced writing paper in an alcoholic solution of lacmoid and drying.

**Cochineal.**—Three grams of cochineal powder are digested with 250 c.c. of a mixture of three parts of water and one part of strong alcohol. The mixture is frequently shaken and at the end of two hours is filtered.

**Günzberg's Reagent.**—Two grams of phloroglucin and one gram of vanillin are dissolved in 100 c.c. of 95 per cent. alcohol and preserved in a dark bottle.

These solutions are conveniently kept in small bottles with ground-in dropping pipettes, one of which is shown in Fig. 191.

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[1] Excellent litmus paper can be obtained commercially, that prepared by Squibb being especially good.

## SPECIFIC GRAVITY TABLES

### POTASSIUM AND SODIUM HYDROXIDE SOLUTIONS AT A TEMPERATURE OF 15° C.

| Specific Gravity | Per cent. of KOH | Per cent. of NaOH | Specific Gravity | Per cent. of KOH | Per cent. of NaOH |
|---|---|---|---|---|---|
| 1.007 | 0.9 | 0.61 | 1.252 | 27.0 | 22.64 |
| 1.014 | 1.7 | 1.20 | 1.263 | 28.0 | 23.67 |
| 1.022 | 2.6 | 2.00 | 1.274 | 28.9 | 24.81 |
| 1.029 | 3.5 | 2.71 | 1.285 | 29.8 | 25.80 |
| 1.036 | 4.5 | 3.35 | 1.297 | 30.7 | 26.83 |
| 1.045 | 5.6 | 4.00 | 1.308 | 31.8 | 27.80 |
| 1.052 | 6.4 | 4.64 | 1.320 | 32.7 | 28.83 |
| 1.060 | 7.4 | 5.29 | 1.332 | 33.7 | 29.93 |
| 1.067 | 8.2 | 5.87 | 1.345 | 34.9 | 31.22 |
| 1.075 | 9.2 | 6.55 | 1.357 | 35.9 | 32.47 |
| 1.083 | 10.1 | 7.31 | 1.370 | 36.9 | 33.69 |
| 1.091 | 10.9 | 8.00 | 1.383 | 37.8 | 34.96 |
| 1.100 | 12.0 | 8.68 | 1.397 | 38.9 | 36.25 |
| 1.108 | 12.9 | 9.42 | 1.410 | 39.9 | 37.47 |
| 1.116 | 13.8 | 10.06 | 1.424 | 40.9 | 38.80 |
| 1.125 | 14.8 | 10.97 | 1.438 | 42.1 | 39.99 |
| 1.134 | 15.7 | 11.84 | 1.453 | 43.4 | 41.41 |
| 1.142 | 16.5 | 12.64 | 1.468 | 44.6 | 42.83 |
| 1.152 | 17.6 | 13.55 | 1.483 | 45.8 | 44.38 |
| 1.162 | 18.6 | 14.37 | 1.498 | 47.1 | 46.15 |
| 1.171 | 19.5 | 15.13 | 1.514 | 48.3 | 47.60 |
| 1.180 | 20.5 | 15.91 | 1.530 | 49.4 | 49.02 |
| 1.190 | 21.4 | 16.77 | 1.546 | 50.6 | .... |
| 1.200 | 22.4 | 17.67 | 1.563 | 51.9 | .... |
| 1.210 | 23.3 | 18.58 | 1.580 | 53.2 | .... |
| 1.220 | 24.2 | 19.58 | 1.597 | 54.5 | .... |
| 1.231 | 25.1 | 20.59 | 1.615 | 55.9 | .... |
| 1.241 | 26.1 | 21.42 | 1.634 | 57.5 | .... |

### AMMONIUM HYDRATE SOLUTIONS AT A TEMPERATURE OF 15° C.

(*Table from* Lunge *and* Wiernik.)

| Specific Gravity | Per cent. of $NH_3$ | Specific Gravity | Per cent. of $NH_3$ | Specific Gravity | Per cent. of $NH_3$ |
|---|---|---|---|---|---|
| 1.000 | 0.00 | 0.960 | 9.91 | 0.920 | 21.75 |
| 0.998 | 0.45 | 0.958 | 10.47 | 0.918 | 22.39 |
| 0.996 | 0.91 | 0.956 | 11.03 | 0.916 | 23.03 |
| 0.994 | 1.37 | 0.954 | 11.60 | 0.914 | 23.68 |
| 0.992 | 1.84 | 0.952 | 12.17 | 0.912 | 24.33 |
| 0.990 | 2.31 | 0.950 | 12.74 | 0.910 | 24.99 |
| 0.988 | 2.80 | 0.948 | 13.31 | 0.908 | 25.65 |
| 0.986 | 3.30 | 0.946 | 13.88 | 0.906 | 26.31 |
| 0.984 | 3.80 | 0.944 | 14.46 | 0.904 | 26.98 |
| 0.982 | 4.30 | 0.942 | 15.04 | 0.902 | 27.65 |
| 0.980 | 4.80 | 0.940 | 15.63 | 0.900 | 28.33 |
| 0.978 | 5.30 | 0.938 | 16.22 | 0.898 | 29.01 |
| 0.976 | 5.80 | 0.936 | 16.82 | 0.896 | 29.69 |
| 0.974 | 6.30 | 0.934 | 17.42 | 0.894 | 30.37 |
| 0.972 | 6.80 | 0.932 | 18.03 | 0.892 | 31.05 |
| 0.970 | 7.31 | 0.930 | 18.64 | 0.890 | 31.75 |
| 0.968 | 7.82 | 0.928 | 19.25 | 0.888 | 32.50 |
| 0.966 | 8.33 | 0.926 | 19.87 | 0.886 | 33.25 |
| 0.964 | 8.84 | 0.924 | 20.49 | 0.884 | 34.10 |
| 0.962 | 9.35 | 0.922 | 21.12 | 0.882 | 34.95 |

## HYDROCHLORIC, NITRIC, AND SULPHURIC ACIDS, AT $\frac{15°}{4°}$ IN[1] A VACUUM. ACCORDING TO G. LUNGE

| Specific Gravity at $\frac{15°}{4°}$ | 100 grams contain | | | Specific Gravity at $\frac{15°}{4°}$ | 100 grams contain | | |
|---|---|---|---|---|---|---|---|
| | HCl | $HNO_3$ | $H_2SO_4$ | | HCl | $HNO_3$ | $H_2SO_4$ |
| 1.000 | 0.16 | 0.10 | 0.09 | 1.175 | 34.42 | 28.63 | 24.12 |
| 1.005 | 1.15 | 1.00 | 0.83 | 1.180 | 35.39 | 29.38 | 24.76 |
| 1.010 | 2.14 | 1.90 | 1.57 | 1.185 | 36.31 | 30.13 | 25.40 |
| 1.015 | 3.12 | 2.80 | 2.30 | 1.190 | 37.23 | 30.88 | 26.04 |
| 1.020 | 4.13 | 3.70 | 3.03 | 1.195 | 38.16 | 31.62 | 26.68 |
| 1.025 | 5.15 | 4.60 | 3.76 | 1.200 | 39.11 | 32.36 | 27.32 |
| 1.030 | 6.15 | 5.50 | 4.49 | 1.205 | | 33.09 | 27.95 |
| 1.035 | 7.15 | 6.38 | 5.23 | 1.210 | | 33.82 | 28.58 |
| 1.040 | 8.16 | 7.26 | 5.96 | 1.215 | | 34.55 | 29.21 |
| 1.045 | 9.16 | 8.13 | 6.67 | 1.220 | | 35.28 | 29.84 |
| 1.050 | 10.17 | 8.99 | 7.37 | 1.225 | | 36.03 | 30.48 |
| 1.055 | 11.18 | 9.84 | 8.07 | 1.230 | | 36.78 | 31.11 |
| 1.060 | 12.19 | 10.68 | 8.77 | 1.235 | | 37.53 | 31.70 |
| 1.065 | 13.19 | 11.51 | 9.47 | 1.240 | | 38.29 | 32.28 |
| 1.070 | 14.17 | 12.33 | 10.19 | 1.245 | | 39.05 | 32.86 |
| 1.075 | 15.16 | 13.15 | 10.90 | 1.250 | | 39.82 | 33.43 |
| 1.080 | 16.15 | 13.95 | 11.60 | 1.255 | | 40.58 | 34.00 |
| 1.085 | 17.13 | 14.74 | 12.30 | 1.260 | | 41.34 | 34.57 |
| 1.090 | 18.11 | 15.53 | 12.99 | 1.265 | | 42.10 | 35.14 |
| 1.095 | 19.06 | 16.32 | 13.67 | 1.270 | | 42.87 | 35.71 |
| 1.100 | 20.01 | 17.11 | 14.35 | 1.275 | | 43.64 | 36.29 |
| 1.105 | 20.97 | 17.89 | 15.03 | 1.280 | | 44.41 | 36.87 |
| 1.110 | 21.92 | 18.67 | 15.71 | 1.285 | | 45.18 | 37.45 |
| 1.115 | 22.86 | 19.45 | 16.36 | 1.290 | | 45.95 | 38.03 |
| 1.120 | 23.82 | 20.23 | 17.01 | 1.295 | | 46.72 | 38.61 |
| 1.125 | 24.78 | 21.00 | 17.66 | 1.300 | | 47.49 | 39.19 |
| 1.130 | 25.75 | 21.77 | 18.31 | 1.305 | | 48.26 | 39.77 |
| 1.135 | 26.70 | 22.54 | 18.96 | 1.310 | | 49.07 | 40.35 |
| 1.140 | 27.66 | 23.31 | 19.61 | 1.315 | | 49.89 | 40.93 |
| 1.145 | 28.61 | 24.08 | 20.26 | 1.320 | | 50.71 | 41.50 |
| 1.150 | 29.57 | 24.84 | 20.91 | 1.325 | | 51.53 | 42.08 |
| 1.155 | 30.55 | 25.60 | 21.55 | 1.330 | | 52.37 | 42.66 |
| 1.160 | 31.52 | 26.36 | 22.19 | 1.335 | | 53.22 | 43.20 |
| 1.165 | 32.49 | 27.12 | 22.83 | 1.340 | | 54.07 | 43.74 |
| 1.170 | 33.46 | 27.88 | 23.47 | 1.345 | | 54.93 | 44.28 |

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[1] The figures indicate that the specific gravity is that obtained at 15° C. compared with the density of water at 4° C.

## HYDROCHLORIC, NITRIC, AND SULPHURIC ACIDS, AT $\frac{15^\circ}{4^\circ}$ IN A VACUUM. ACCORDING TO G. LUNGE (*Continued*)

| Specific Gravity at $\frac{15^\circ}{4^\circ}$ in a vacuum | 100 grams contain | | Specific Gravity at $\frac{15^\circ}{4^\circ}$ in a vacuum | 100 grams contain | Specific Gravity at $\frac{15^\circ}{4^\circ}$ in a vacuum | 100 grams contain |
|---|---|---|---|---|---|---|
| | $HNO_3$ | $H_2SO_4$ | | $H_2SO_4$ | | $H_2SO_4$ |
| 1.350 | 55.79 | 44.82 | 1.540 | 63.43 | 1.730 | 79.80 |
| 1.355 | 56.66 | 45.35 | 1.545 | 63.85 | 1.735 | 80.24 |
| 1.360 | 57.57 | 45.88 | 1.550 | 64.26 | 1.740 | 80.68 |
| 1.365 | 58.48 | 46.41 | 1.555 | 64.67 | 1.745 | 81.12 |
| 1.370 | 59.39 | 46.94 | 1.560 | 65.08 | 1.750 | 81.56 |
| 1.375 | 60.30 | 47.47 | 1.565 | 65.49 | 1.755 | 82.00 |
| 1.380 | 61.27 | 48.00 | 1.570 | 65.90 | 1.760 | 82.44 |
| 1.385 | 62.24 | 48.53 | 1.575 | 66.30 | 1.765 | 82.88 |
| 1.390 | 63.23 | 49.06 | 1.580 | 66.71 | 1.770 | 83.32 |
| 1.395 | 64.25 | 49.59 | 1.585 | 67.13 | 1.775 | 83.90 |
| 1.400 | 65.30 | 50.11 | 1.590 | 67.59 | 1.780 | 84.50 |
| 1.405 | 66.40 | 50.63 | 1.595 | 68.05 | 1.785 | 85.10 |
| 1.410 | 67.50 | 51.15 | 1.600 | 68.51 | 1.790 | 85.70 |
| 1.415 | 68.63 | 51.66 | 1.605 | 68.97 | 1.795 | 86.30 |
| 1.420 | 69.80 | 52.15 | 1.610 | 69.43 | 1.800 | 86.90 |
| 1.425 | 70.98 | 52.63 | 1.615 | 69.89 | 1.805 | 87.60 |
| 1.430 | 72.17 | 53.11 | 1.620 | 70.32 | 1.810 | 88.30 |
| 1.435 | 73.39 | 53.59 | 1.625 | 70.74 | 1.815 | 89.05 |
| 1.440 | 74.68 | 54.07 | 1.630 | 71.16 | 1.820 | 90.05 |
| 1.445 | 75.98 | 54.55 | 1.635 | 71.57 | 1.825 | 91.00 |
| 1.450 | 77.28 | 55.03 | 1.640 | 71.99 | 1.830 | 92.10 |
| 1.455 | 78.60 | 55.50 | 1.645 | 72.40 | 1.835 | 93.43 |
| 1.460 | 79.98 | 55.97 | 1.650 | 72.82 | 1.840 | 95.60 |
| 1.465 | 81.42 | 56.43 | 1.655 | 73.23 | 1.8405 | 95.95 |
| 1.470 | 82.90 | 56.90 | 1.660 | 73.64 | 1.8410 | 97.00 |
| 1.475 | 84.45 | 57.37 | 1.665 | 74.07 | 1.8415 | 97.70 |
| 1.480 | 86.05 | 57.83 | 1.670 | 74.51 | 1.8410 | 98.20 |
| 1.485 | 87.70 | 58.28 | 1.675 | 74.97 | 1.8405 | 98.70 |
| 1.490 | 89.60 | 58.74 | 1.680 | 75.42 | 1.8400 | 99.20 |
| 1.495 | 91.60 | 59.22 | 1.685 | 75.86 | 1.8495 | 99.45 |
| 1.500 | 94.09 | 59.70 | 1.690 | 76.30 | 1.8390 | 99.70 |
| 1.505 | 96.39 | 60.18 | 1.695 | 76.73 | 1.8385 | 99.95 |
| 1.510 | 98.10 | 60.65 | 1.700 | 77.17 | | |
| 1.515 | 99.07 | 61.12 | 1.705 | 77.60 | | |
| 1.520 | 99.67 | 61.59 | 1.710 | 78.04 | | |
| 1.525 | | 62.06 | 1.715 | 78.48 | | |
| 1.530 | | 62.53 | 1.720 | 78.92 | | |
| 1.535 | | 63.00 | 1.725 | 79.36 | | |

## ALCOHOL AT A TEMPERATURE OF 15.6° C.

| Specific Gravity | Per cent. by Volume | Per cent. by Weight |
|---|---|---|
| 0.8941 | 68 | 60.38 |
| 0.8917 | 69 | 61.42 |
| 0.8892 | 70 | 62.50 |
| 0.8867 | 71 | 63.58 |
| 0.8842 | 72 | 64.66 |
| 0.8817 | 73 | 65.74 |
| 0.8791 | 74 | 66.83 |
| 0.8765 | 75 | 67.93 |
| 0.8739 | 76 | 69.05 |
| 0.8712 | 77 | 70.18 |
| 0.8685 | 78 | 71.31 |
| 0.8658 | 79 | 72.45 |
| 0.8631 | 80 | 73.59 |
| 0.8603 | 81 | 74.74 |
| 0.8575 | 82 | 75.91 |
| 0.8547 | 83 | 77.09 |
| 0.8518 | 84 | 78.29 |
| 0.8488 | 85 | 79.50 |
| 0.8458 | 86 | 80.71 |
| 0.8428 | 87 | 81.94 |
| 0.8397 | 88 | 83.19 |
| 0.8365 | 89 | 84.46 |
| 0.8332 | 90 | 85.75 |
| 0.8299 | 91 | 87.06 |
| 0.8265 | 92 | 88.37 |
| 0.8230 | 93 | 89.71 |
| 0.8194 | 94 | 91.07 |
| 0.8157 | 95 | 92.46 |
| 0.8118 | 96 | 93.89 |
| 0.8077 | 97 | 95.34 |
| 0.8034 | 98 | 96.84 |
| 0.7988 | 99 | 98.39 |
| 0.7939 | 100 | 100.00 |

## NORMAL SOLUTIONS

A normal solution of a substance is one which contains the hydrogen equivalent of its molecular weight dissolved in a liter of water, hydrogen being considered for analytical purposes as 1, though in the following table of atomic weights it is given as 1.008.

Equal volumes of normal solutions should combine exactly with each other; for example, 1 c.c. of $^{N}/_{1}$ hydrochloric acid should exactly neutralize 1 c.c. of $^{N}/_{1}$ sodium or potassium hydrate or 10 c.c. of $^{N}/_{10}$ alkali.

Atomic weights of the elements most frequently used in making normal solutions are as follows:

| | | |
|---|---|---|
| Barium | Ba | 137.4 |
| Bromine | Br | 79.9 |
| Calcium | Ca | 40.1 |
| Carbon | C | 12. |
| Chlorine | Cl | 35.45 |
| Copper | Cu | 63.6 |
| Hydrogen | H | 1.008 |
| Iodine | I | 126.85 |
| Iron | Fe | 55.9 |
| Lead | Pb | 206.92 |
| Magnesium | Mg | 24.3 |
| Manganese | Mn | 55. |
| Mercury | Hg | 200. |
| Nitrogen | N | 14.04 |
| Oxygen | O | 16. |
| Potassium | K | 39.11 |
| Silver | Ag | 107.92 |
| Sodium | Na | 23.05 |
| Sulphur | S | 32.07 |
| Tungsten | W | 184. |
| Uranium | U | 239.6 |
| Zinc | Zn | 65.4 |

A normal solution of sodium hydroxide (NaOH) contains Na, 23.05; O, 16; H, 1.008 = 40.058 grams to the liter of water. For ordinary purposes the decimal portion of this figure may be omitted and the amount considered as 40 grams.

Dibasic acids or salts of these acids contain only half the molecular weight in grams to the liter. For example, normal sulphuric acid contains 49.04 grams to the liter, instead of 98.08, its molecular weight. Normal sodium carbonate, also, contains 53 grams to the liter instead of 106.

The following normal solutions are the ones chiefly used in the clinical laboratory:

| | | | |
|---|---|---|---|
| Oxalic acid | $C_2O_4H_2, 2H_2O$ | 63.024 | grams per liter |
| Hydrochloric acid | $HCl$ | 36.46 | " " |
| Sulphuric acid | $\left\{\frac{H_2SO_4}{2}\right\}$ | 49.04 | " " |
| Sodium hydrate | $NaOH$ | 40.058 | " " |
| Potassium hydrate | $KOH$ | 56.16 | " " |
| Ammonium hydrate | $NH_3$ | 17.064 | " " |
| Sodium carbonate | $\left\{\frac{Na_2CO_3}{2}\right\}$ | 53.05 | " " |
| Silver nitrate | $AgNO_3$ | 169.97 | " " |
| | $Ag$ | 107.92 | " " |
| Silver nitrate[1] | $\left\{\frac{AgNO_3}{5.85}\right\}$ | 29.06 | " " |
| | $Ag$ | 18.449 | " " |
| Sodium chloride | $NaCl$ | 58.5 | " " |
| Potassium permanganate | $\left\{\frac{KMnO_4}{5}\right\}$ | 31.63 | " " |

**Normal Sodium Hydrate Solution.**—As normal sodium hydrate[2] is the solution most often required in the clinical laboratory, its preparation will be given in full.

Accurate normal solutions of sodium hydrate can not be made by weighing out that substance, because it attracts water from the air, thus constantly changing in weight, and also because of the difficulty of handling such a corrosive solid. Commercial sodium hydrate also contains carbonate and sulphate, which reduces its alkalinity.

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[1] A special silver nitrate solution is often used, 1 c.c. of which is equivalent to .01 grams of sodium chloride. This obviates complicated multiplication in Volhard's method in determining chlorides in the urine or HCl in stomach contents.

[2] Unless the practitioner has access to a laboratory it is best to purchase the only normal solution which he will ordinarily use, i. e., normal sodium hydrate. This is to be kept in a closely stoppered bottle and diluted with nine volumes of water when needed. Accurate normal solutions may be obtained from Eimer & Amend, New York.

For clinical purposes, Merck's "Sodium hydrate by alcohol" is sufficiently pure to give a closely approximate normal solution if 41 grams are dissolved in water and the graduate filled up to a liter. Great care must be taken, however, that the sticks of sodium hydrate are protected from the air in a bottle, the glass stopper of which has been sealed with vaselin. A few days' exposure to the air will give rise to a considerable amount of sodium carbonate on the surface of the sticks, thus giving an inaccurate solution.

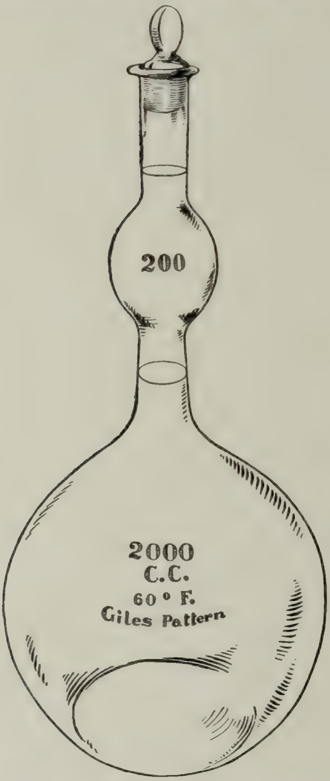

Fig. 192.—Giles Pattern Measuring Flask.

For more accurate work, normal solutions are best obtained by first preparing a standard normal solution of some acid and then using this to determine the strength of the sodium hydrate. For this purpose the most convenient acid is oxalic.

Chemically pure oxalic acid crystals can be obtained from Merck, and are readily weighed on a balance, as the substance is not deliquescent. The molecular weight of oxalic acid is 126, but as it is dibasic, the amount required for a normal solution is one-half of this amount; in other words, 63 grams in 1,000 c.c. of water. It is more economical to use 6.3 grams and dissolve in 100 c.c. of water.

In making up an approximately normal solution it is convenient to use a Giles measuring flask. This has two graduations on the neck, one at 1,000 c.c., the other at 1,100 c.c., thus giving an extra 100 c.c. to be titrated in standardizing the solution (Fig. 192). An extra quantity of NaOH should be used, about 46 grams to 1,100 c.c. Ten c.c. of the normal acid solution are carefully measured off by means of a pipette, a few drops of phenolphthalein added, and the mixture titrated with sodium hydrate solution which has been made up approximately by weight, until a faint pink color appears, which remains permanent after stirring. If the sodium hydrate solution is accurately normal, 10 c.c. will neutralize 10 c.c. of the oxalic acid. If, however, only 9 c.c. of the sodium hydrate is required to neutralize the acid, the sodium hydrate is evidently 10 per cent. too strong, and 1 c.c. of distilled water should be added to each 9 c.c., in the measuring flask. The flask is inverted after the addition of the water, so as to mix the solutions thoroughly, the burette is washed out first with distilled water and then with a little of the normal solution, which has been corrected by dilution, and finally filled with that solution.

A fresh portion of the oxalic acid is measured out in a beaker, phenolphthalein added, and the titration repeated. The second result should be close to the required amount; that is, it should take 9.8 to 9.9 c.c. of the sodium hydrate to change the indicator to the faint pink color. It is well at this stage to titrate with a larger quantity of acid, using, for example, 50 c.c., before making the final dilution which is necessary to bring the sodium hydrate solution exactly to the normal.

In another procedure which furnishes more accurate results a weighed amount of sodium carbonate is used to estimate the strength of a standard acid. By titrating this standard acid against the unknown sodium hydrate solution, the exact strength of the latter can be obtained, and the solution diluted accordingly. About 8 grams of Merck's chemically pure sodium bicarbonate is placed in a large platinum crucible and heated over an alcohol flame. The contents of the crucible should be continuously stirred with the end of a platinum wire which remains in the crucible and is weighed with it. The heating should continue for about half an hour to drive off the $CO_2$ and form $Na_2CO_3$, the temperature never exceeding a dull red heat at the bottom of the crucible. Any melting or caking of the sodium bicarbonate should be avoided, as under these conditions carbonic acid may be driven off and sodium hydrate formed. After the heating has been continued for about half an hour, the crucible is cooled in an exsiccator and weighed. The heating is repeated for a short time and a second weighing is made. If the results agree, about 2 grams of the dried sodium carbonate are shaken out of the crucible into a beaker, the amount used being determined by weighing the crucible and its contents. The carbonate is dissolved in about 100 c.c. of distilled water. Five to six drops of methyl-orange solution are added, and approximately normal hydrochloric acid or sulphuric acid is run in from a burette until a drop of acid gives rise to a pure rose color. Assuming that 39.20 c.c. of hydrochloric acid are used to neutralize 2.1132 grams of sodium carbonate, the strength of the acid may be computed as follows.[1]

If the acid had been exactly normal, 1,000 c.c. of it would have neutralized 53.05 grams of sodium carbonate. For the molecular weight of the latter is 106.10, and as carbonic acid is dibasic the normal weight would be $\frac{106.10}{2} = 53.05$. The 2.1132 grams of sodium carbonate should therefore have required 39.83 c.c. of normal acid to neutralize it according to the equation—

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[1] *Treadwell:* Lehrbuch d. analytischen Chemie, Bd. ii, 1903, p. 384.

$$53.05 : 1{,}000 : : 2.1132 : X.$$

$$X = \frac{2113.2}{53.05} = 39.83.$$

Since, however, only 39.20 c.c. were used, the acid is evidently too strong, and to every 39.20 c.c., 0.63 c.c. of water should be added to make it normal. Therefore, to one liter 16.07 c.c. of water should be added.

A dry liter flask is exactly filled to the mark with the acid and 16.07 c.c. of water allowed to run in from a burette or an exactly divided pipette. The contents of the flask are then thoroughly mixed. Approximately 2 grams of sodium carbonate are again weighed out, dissolved in 100 c.c. of distilled water, methyl orange is added, and a second titration with the diluted acid is carried out. A second dilution may be made if the acid still proves too strong, or the flask may be marked with the exact equivalent of the acid and the results multiplied by the corresponding factor.

For clinical use normal sodium hydrate is too strong, so that it is always diluted to $^1/_{10}$ or $^1/_{20}$ normal. For this purpose, 50 or 100 c.c. may be measured off by a pipette into a measuring flask, and the latter filled up to the mark 500 or 1,000, care being taken that the temperature of the fluid at the time of mixing is the same as that marked on the flask.

**Decinormal Oxalic Acid.**—The molecular weight of oxalic acid is 126.024, and as the acid is dibasic, a decinormal solution would contain one-twentieth of the molecular weight; that is, 6.3012 grams. For ordinary work the commercial acid may be assumed as sufficiently pure to use as standard.

For especially accurate determinations, however, the acid should be purified by heating a mixture of 500 grams of this substance and 500 grams of pure hydrochloric acid of a specific gravity of 1.075 in a porcelain dish. The solution is filtered and the filtrate collected in a crystallizing vessel and placed on ice in order to cool it as quickly as possible. The oxalic acid crystallizes out in fine crystalline powder, and should be freed from the excess of acid by pouring the mixture into a funnel, the lower portion of which is closed by a perforated platinum cone. The oxalic acid is again dissolved in a small quantity of boiling hydrochloric acid, separated by crystallization, and dried. The crystals are washed twice in small quantities of ice-cold water, crystallized three times out of hot water, and may then be considered as chemically pure.

**Decinormal Potassium Permanganate Solution.**—This solution can not be made up by direct weighing, but must be titrated against decinormal solution of oxalic acid. Twenty-five c.c. of the oxalic solution are placed in a beaker, 10 c.c. of 25 per cent. sulphuric acid are added, and the whole is diluted with water at a temperature of 70° C. to about

200 c.c. The permanganate solution to be standardized is run in from a burette with a glass stop-cock and the mixture constantly stirred. As soon as all the oxalic acid is oxidized the red color is permanent.

Decinormal potassium permanganate solution contains 3.163 grams to the liter, the decomposition of the salt taking place according to the formula $K_2O.Mn_2O_7 = K_2O + 2MnO + O_5$. If Kahlbaum's chemically pure potassium permanganate is used, solutions can be made up of sufficient accuracy to use without control titration, at least for such analyses as the quantitative determination of uric acid in the urine according to Folin. As a rule, however, commercial permanganate is very impure, and, as stated above, must be titrated against oxalic acid. The oxalic acid solution for this purpose keeps much better if it contains 50 c.c. of concentrated sulphuric acid to the liter. After eight months' keeping such a solution changes its titer about .12 per cent.[1]

**Potassium Sulphocyanide.**—A solution of potassium sulphocyanide is used in the determination of the chlorides in the urine and other fluids by the Volhard method. A decinormal solution, containing 9.72 grams to the liter, can be used, or a special solution containing 16.6 grams of the sulphocyanide, which corresponds to a special silver nitrate solution 1 c.c. of which is equivalent to 1 centigram of sodium chloride.

As the potassium sulphocyanide is hygroscopic, a standard solution can not be made up by direct weighing, but only by titration against a previously prepared standard silver solution. As the preparation of both solutions is similar in principle, only one will be described. The reaction between silver nitrate and potassium sulphocyanide is $AgNO_3 + KSCN = AgSCN + KNO_3$. In other words, one molecule of silver nitrate weighing 169.97, is precipitated by a molecule of potassium sulphocyanide weighing 97.22. As the silver nitrate solution corresponding to 1 centigram of sodium chloride contains 29.06 grams to the liter, the sulphocyanide solution which corresponds to this should be 16.6 grams to the liter. For the special silver nitrate solution corresponding to 1 centigram of sodium chloride contains 169.97, divided by 5.85, which equals 29.6 grams to the liter, so the corresponding potassium sulphocyanide solution would contain 97.22 divided by 5.85, which equals 16.6 grams to the liter.

About 18 grams of the sulphocyanide are weighed out and dissolved in about 900 c.c. of water. Ten c.c. of the standard silver solution are diluted to 100 with water. Four c.c. of nitric acid and 5 c.c. of the ammonio-ferric alum are added and the mixture is titrated with the potassium sulphocyanide of unknown strength. The end reaction is marked by the production of a slight red color which remains on stirring the fluid. Inasmuch as the sulphocyanide solution has been purposely made too strong, less than 10 c.c. will be required to neutralize 10 c.c. of the silver solution. If we assume that 9.8 c.c. of the sulphocyanide are used, then it is evident that to each 9.8 c.c. of the solution, 10 minus 9.8 c.c. or 0.2 c.c. of water should be added to bring the solution to the proper strength. Nine hundred and eighty c.c. of the fluid are measured off and filled up to a thousand with distilled water. Or, 1,000 c.c. are measured off and 24.08 c.c. of distilled water are added from a burette. The contents of the flask are thoroughly shaken; a fresh sample is withdrawn and titrated against another 10 c.c. of silver nitrate. The second dilution will probably require only the addition of a few c.c. of water in order to bring it to the standard. If preferred, however, the solution may be left of an arbitrary strength and the correction made at the time of titrating the urine.

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[1] *Treadwell:* Analytische Chemie, Bd. ii, Leipzig, 1903.

**Decinormal Sodium Thiosulphate.**—A decinormal thiosulphate solution can not be produced directly by weighing out the crystalline salt, but must be standardized against an iodine solution of known strength. The reaction takes place according to the following formula: $2Na_2S_2O_3 + I_2 = 2NaI + Na_2S_4O_6$. Commercial iodine "resublimed" is pure enough for clinical purposes. For accurate technical analyses the iodine should be sublimed by heating about 6 grams of this substance with 2 grams of pure potassium iodide in order to remove chlorine and bromine, which are often present. The iodine is placed at the bottom of a small beaker and a flask containing cold water is allowed to rest on the rim of the beaker. The iodine as it sublimes collects in the form of a crust on the under surface of the flask. In order to weigh out the iodine a small weighing bottle is used containing one-half c.c. of water and 2 grams of pure potassium iodide. The bottle and its contents are carefully weighed, the balance arrested, and 4 or 5 centigrams of pure iodine thrown into the bottle and the stopper immediately inserted. The second weighing gives the amount of iodine added to the contents of the flask. The weighing bottle and its contents are dropped into a wide-mouthed Erlenmeyer flask, the stopper being withdrawn as the bottle is allowed to fall. The flask should contain about 200 c.c. of water and 1 gram of potassium iodide. The flask is filled up to 500 c.c.

This iodine solution is then used to standardize the sodium thiosulphate solution which has already been made up by weighing out 25 grams of the crystallized salt ($Na_2S_2O_3 + 5H_2O$; molecular weight, 248.32). The sodium thiosulphate solution should be prepared some two weeks before it is titrated, as the carbon dioxide in the distilled water rapidly alters the titer by decomposing the salt; but after the end of two weeks all of the $CO_2$ is removed and the solution remains permanent for six or eight months.

A measured quantity of the iodine solution is placed in a beaker. Two or three c.c. of thin starch paste are added and the sodium thiosulphate solution run in until the blue of the iodine has disappeared. The titration is repeated once or twice and then the thiosulphate diluted by the necessary amount of water, which has previously been thoroughly boiled; or, what is more satisfactory, the strength of the solution is determined and no attempt made to bring it to the decinormal point; but the iodine capacity of each c.c. is noted and the results corrected accordingly.

If, for example, 1 c.c. of the sodium thiosulphate solution is equivalent to 0.01167 grams of iodine, this figure should be divided by the amount of iodine contained in a true normal solution—that is, 0.01268. The result, 0.9201 is the factor by which each c.c. of the sodium thiosulphate solution should be multiplied in order to be changed into an accurate decinormal. If desired, a portion of this thiosulphate solution may be diluted to a true normal and an iodine solution standardized, but as the iodine only keeps for a few days it is better to use the standard thiosulphate and make a correction for each determination.[1]

**Silver Nitrate Solution.**—Either 10.792 grams or 18.449 grams (see foot-note, p. 741) of pure silver foil are dissolved in nitric acid, the excess of acid driven off by boiling and the solution diluted to 1,000 c.c.

## GLYCERIN JELLY

Glycerin jelly is useful for preserving casts, crystals, and parasites. Kaiser's formula.—Seven grams of clear gelatin are soaked for two hours in 42 c.c. of distilled water. Fifty grams of glycerin and one gram of phenol are added and the whole warmed on a water bath until the gelatin is dissolved. The mixture should be constantly stirred while being heated, and as soon as it is perfectly fluid it should be filtered, hot, through either paper or glass wool.

## DETERMINATION OF THE MELTING POINT

The determination of melting points has its chief interest in medicine in the identification of the various osazons derived from the sugars excreted in the urine or contained in the exudates of the body. The osazon or other substance of which the melting point is to be determined is dried and finely powdered. A small quantity of the powder is then shaken into a capillary tube and pressed into the tip by a fine wire or glass thread. The tubes are made by drawing out thin glass tubing into capillary tubes of a diameter of about 1 to 2 millimeters and sealing one end. After the tube is filled it is fastened to the stem of an accurate thermometer by means of a few turns of platinum wire or by a short section of rubber tubing of such a diameter that the tube is held firmly in place with the end containing the powder at the level of the thermometer bulb. The tube and thermometer are then heated in a suitable fluid. For crotonic acid, water affords sufficient heat; for the osazons, strong sulphuric acid is necessary. Unless the acid is perfectly free from organic matter it will turn brown as soon as heat is applied. To obviate this, a crystal of potassium nitrate or permanganate should be dropped into the acid to aid in the oxidation of the carbon and thus render the fluid perfectly clear. The acid may be contained in a long-necked flask or even in a beaker and should be in sufficient quantity to cover entirely the bulb of the thermometer. The fluid is to be rapidly heated to a point about ten degrees below the melting point, and then very gradually until the small mass of powder is suddenly seen to become clear and show a meniscus. In the case of the osazons, the powder suddenly becomes black and opaque, and the fluid clings to the walls of the capillary. If the approximate melting point is not known, the substance should be slowly heated and small particles in the upper portion of the capillary tube carefully watched. The hot acid rising to the top will melt these fragments before that portion which is close to the bulb of the thermometer becomes fluid. If the temperature is now carefully raised for about a degree, the powder at the level of the bulb will be noticed to melt. The height of the thermometer is then read off. A second and more careful trial will usually give a point one or two degrees below that first obtained, as the temperature can be more carefully raised when the approximate melting point is known. For ordinary purposes it is not necessary to correct for differences in barometric pressure. The usual error of the method is ± 0.2° to 0.5°.[1]

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[1] *Treadwell:* l. c., vol. ii, p. 447.

## CLEANING SLIDES AND COVER GLASSES

Slides and cover glasses which have not been previously used and are not very greasy may be cleaned by prolonged immersion in strong alcohol, containing about 20 per cent. of strong hydrochloric acid. After several weeks soaking in this mixture, the alcohol may be poured off and replaced by fresh alcohol to remove the acid. The slides or covers may then be dried and put in suitable boxes.

In cleaning slides or covers which are much soiled, or which have been used before, it is most convenient to employ strong hot nitric acid. Take a large beaker and place in it several ounces of covers, or a gross of slides, then pour over them a few cubic centimeters of strong alcohol. The alcohol quickly penetrates between the slides, despite the grease with which they are covered. The alcohol is drained off as completely as possible, and the beaker placed beneath a hood or out of doors. About 50 c.c. of strong nitric acid is then poured over the slides. In a few seconds a sharp reaction between the acid and the alcohol will take place, with the evolution of reddish-brown fumes of nitric peroxide and violent boiling of the nitric acid. Care should be taken that none of the acid is spurted over the face or hands of the operator. The heat and boiling of the acid cause it to penetrate between the individual slides and replace the alcohol which is being oxidized, so that the cleansing process takes place thoroughly and quickly. In a few minutes the acid can be washed away with tap water, followed by distilled water until all acid reaction has disappeared; then alcohol, and finally ether. If the ether is perfectly pure and gives no residue on evaporation, the covers can be simply allowed to dry in the air; if, however, the ether does leave a residue, as is usually the case with the commercial product, the covers are best dried by spreading out on a towel and wiping off each cover as it lies flat on the cloth surface. A small wooden block covered with clean cloth can be used very conveniently to rub off the covers as they lie flat on the surface of the towel. If any attempt is made to hold the covers between the fingers while rubbing them off with a cloth, so many are broken that the process becomes a very expensive one. Slides, of course, can be handled more roughly, and need not be treated with the ether, but can be dried by simply wiping off the alcohol in which they are kept.

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[1] *Ostwald-Luther:* Physiko-Chem. Messungen, 1902.

## REMOVING ANILINE DYES FROM THE HANDS

In order to clean the hands after staining with aniline dyes it is necessary to use different procedures for the different colors. If the stain contains an $HN_2$ group, as does fuchsin, it is possible to remove the dye by washing in a faintly acid solution of sodium nitrite. The coloring matter is changed to a corresponding diazo compound which is colorless and can be removed by washing in water. This procedure will also remove fuchsin from clothing if the latter is allowed to soak for some time in the nitrite solution. This method will not completely remove methylene blue, gentian violet or eosin, but a scheme which is generally applicable for removing any dye is as follows:

Dip the hands into a warm solution of potassium permanganate which has been slightly acidulated with sulphuric acid and allow them to remain several minutes, by which means the coloring matter is oxidized and destroyed. After washing off the excess of permanganate, the brown stain of the manganese dioxide is removed by washing the hands in a saturated solution of oxalic acid or in a solution of sulphurous acid.

Another method of removing aniline dyes is to rub over the hands a thick paste made by mixing bleaching powder and crystals of washing soda ($Na_2CO_3$). The decomposition of the chloride of lime by the sodium carbonate sets free an actively oxidizing compound, hypochlorous acid, which destroys the coloring matter. The hands are then freed from the acid by washing in water.

## MAKING A GOOCH FILTER

A Gooch crucible filter is so convenient and generally employed in gravimetric analysis that the method of its manufacture is given here.

A suspension of asbestos fiber in water is poured into the crucible while strong suction is being applied, so that a firm, felt work is formed. The asbestos is prepared by scraping the crude material with a knife and adding the fibers to a large bulk of 5 per cent. hydrochloric acid in a tall cylinder. Air is blown through to separate the fibers thoroughly, and the mixture is allowed to settle for a few minutes. The upper portion of the fluid containing the finer fibers is decanted and kept separately from the lower. In making the filter the coarse material is poured on first and a little of the fine afterward. The filter is then washed by drawing distilled water through in a slow stream, dried at 120° C., ignited, allowed to cool for half an hour in an exsiccator, and weighed.

In igniting the barium sulphate precipitates the flame must not be applied directly to the bottom of the crucible, in order to avoid mechanical losses. The crucible is placed on a platinum crucible lid on a pipe-stem triangle, and the outer, oxidizing flame of the Bunsen burner is used, first gently, and finally full force.

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